UNIVERSITY OF ALBERTA

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL TRICYCLIC CYCLOOXYGENASE-2 (COX-2) INHIBITORS

BY

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requirements for the degree of DOCTOR OF PHILOSOPHY

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Dedicated to my family;

Dad, Mom, Brother, Sister-in-Law and my Wife

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TABLE OF CONTENTS

1.0.0.0.	**INTRODUCTION**.....	1
1.1.0.0.	THE CYCLOOXYGENASES.....	2
1.2.0.0.	THE CYCLOOXYGENASE STRUCTURE.....	2
1.2.1.0.	Cyclooxygenase (COX) and peroxidase (POX) activities...	3
1.2.1.1.	Comparisons of COX-1 and COX-2.....	4
1.2.1.2.	Structural differences between COX-1 and COX-2.....	5
1.2.1.3.	Regulation and expression of COX-1 and COX-2.....	6
1.2.1.4.	Role of COX enzymes in inflammation.....	6
1.2.1.5.	COX enzymes in the gastrointestinal system.....	7
1.2.1.6.	COX enzymes in the kidney.....	7
1.2.1.7.	COX enzymes and the cardiovascular system.....	8
1.3.0.0.	SELECTIVE CYCLOOXYGENASE-2 (COX-2) INHIBITORS	8
1.3.1.0.	CHEMICAL CLASSIFICATION OF SELECTIVE COX-2 INHIBITORS.....	10
1.3.1.1.	Methanesulfonanilides.....	10
1.3.1.2.	Diarylheterocycles as selective COX-2 inhibitors.....	11
1.3.1.3.	Diarylheterocycles with a central 5-membered thiophene ring.	11
1.3.1.4.	Diarylheterocycles with a central 5-membered pyrazole ring...	12
1.3.1.5.	Diarylheterocycles with a central 5-membered furanone ring....	12
1.3.1.6.	Diarylheterocycles with a central 5-membered isoxazole ring....	13
1.3.1.7.	Diarylheterocycles with a central 6-membered pyridine ring....	14
1.3.1.8.	Diarylheterocycles with a central 6-membered pyridazinone ring.	14

1.3.1.9.	Diarylheterocycles with a central 6-membered pyranone ring.	15
1.3.2.0.	Diarylcarbocycles as selective COX-2 inhibitors.....	15
1.3.2.1.	Diarylcarbocycles with a central 4-membered cyclobutene or cyclobutanone ring.....	15
1.3.2.2	Diarylcarbocycles with a central 5-membered cyclopentene or cyclopentenone ring.....	16
1.3.2.3.	Diarylcarbocycles with a central 6-membered benzene ring system	16
1.4.0.0.	MOLECULAR MODELING STUDIES OF SELECTIVE CYCLOOXYGENASE-2 (COX-2) INHIBITORS.....	17
1.4.1.0.	Structural basis for COX inhibition by NSAIDs and selective COX-2 inhibitors.....	17
1.4.1.1.	Examples of successful application of molecular modeling (docking) experiments in the design of selective COX-2 inhibitors.....	19
1.4.1.2.	Design of COX-2 selectivity into the nonselective COX inhibitor flurbiprofen.....	19
1.4.1.3.	Design of COX-2 selectivity into the nonselective COX inhibitor indomethacin.....	20
1.4.1.4.	Replacement of sulfonamide and methylsulfone pharmacophores in celecoxib and rofecoxib by an azido bioisostere.....	20
1.5.0.0.	THERAPEUTIC APPLICATION OF SELECTIVE COX-2 INHIBITORS.....	21
1.5.1.0.	Arthritis.....	21
1.5.1.1.	Cancer.....	21

1.5.1.2.	Alzheimer's disease (AD).....	22
1.5.1.3.	Parkinson's disease (PD).....	22
1.6.0.0.	REFERENCES.....	23
1.7.0.0.	OBJECTIVES OF THE RESEARCH.....	34
2.0.0.0.	**CHAPTER 1.0:** Design, syntheses and biological evaluation of novel 1,1-dihalo-2,3-diphenylcyclopropanes as potential cyclooxygenase-2 (COX-2) inhibitors with antiinflammatory-analgesic activity.....	35
2.1.0.0.	Introduction.....	35
2.2.0.0.	Chemistry.....	36
2.3.0.0.	Results and discussion.....	37
2.4.0.0.	Conclusions.....	41
2.5.0.0.	Experimental Section.....	42
2.6.0.0.	References.....	48
2.7.0.0.	Appendix 1.1.....	52
3.0.0.0.	**CHAPTER 2.0:** 6-Alkyl, alkoxy, or alkylthio-substituted 3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones: a novel class of diarylhetero-cyclic selective cyclooxygenase-2 inhibitors.....	53
3.1.0.0.	Introduction.....	53
3.2.0.0.	Chemistry.....	53
3.3.0.0.	Results and discussion.....	55
3.4.0.0.	Conclusions.....	57

3.5.0.0.	References.....	57
4.0.0.0.	**CHAPTER 3.0:** Design, synthesis and biological evaluation of 6-alkyl, alkoxy or alkylthio-substituted-3-(4-methane sulfonylphenyl)-4-phenylpyran-2-ones: A novel class of diaryl heterocyclic selective cyclooxygenase-2 inhibitors.....	61
4.1.0.0.	Introduction.....	61
4.2.0.0.	Chemistry.....	62
4.3.0.0.	Results and discussion.....	64
4.4.0.0.	Conclusions.....	69
4.5.0.0.	Experimental Section.....	69
4.6.0.0.	References.....	79
4.7.0.0.	Appendix 3.1.....	84
5.0.0.0.	**CHAPTER 4.0:** Design, synthesis and structure-activity relationship (SAR) studies of 3,4,6-triphenylpyran-2-ones as selective cyclo-oxygenase-2 inhibitors.....	86
5.1.0.0.	Introduction.....	86
5.2.0.0.	Chemistry.....	87
5.3.0.0.	Results and discussion.....	91
5.4.0.0.	Conclusions.....	100
5.5.0.0.	Experimental Section.....	100
5.6.0.0.	References.....	125
5.7.0.0.	Appendix 4.1.....	129

6.0.0.0.	**GENERAL DISCUSSION AND CONCLUSIONS**.....	134
6.1.0.0.	Design, syntheses, and evaluation of novel 1,1-dihalo-2,3-diphenylcyclopropanes as potential cyclooxygenase-2 (COX-2) inhibitors with antiinflammatory-analgesic activity....	134
6.2.0.0.	Design, synthesis and biological evaluation of 6-alkyl, alkoxy or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenyl pyran-2-ones: A novel class of diarylheterocyclic selective cyclooxygenase-2 inhibitors.....	135
6.3.0.0.	Design, synthesis and structure-activity relationship (SAR) studies of 3,4,6-triphenylpyran-2-ones as selective cyclo-oxygenase-2 inhibitors.....	138
6.4.0.0.	Molecular modeling studies of isomeric acetoxy analogs of rofecoxib.....	141
6.5.0.0	Molecular modeling studies of celecoxib analogs.....	143
6.6.0.0.	Conclusions.....	146
6.7.0.0.	REFERENCES.....	146

LIST OF SCHEMES

CHAPTER 1.0

Scheme 1.1: The synthesis of (*Z*)- or (*E*)-stilbenes (**8a–e** and **9a–e**)..... 36

Scheme 1.2: The synthesis of (*Z*)- or (*E*)-1,1-dihalo-2,3-diphenylcyclopropanes (**10a–d** and **11a–d**)..... 37

Scheme 1.3: The synthesis of (*Z*)- or (*E*)-1,1-dihalo-2,3-diphenylcyclopropanes (**10e–l** and **11e–l**)..... 37

CHAPTER 2.0

Scheme 2.1: The synthesis of 2,3-diphenylcyclopropenone (**6**)..... 54

Scheme 2.2: The synthesis of pyridinium salts (**9a–i**)..... 54

Scheme 2.3: The synthesis of 6-alkyl, alkoxy or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**10a–c**, **11a–c** and **12a–c**)..... 54

CHAPTER 3.0

Scheme 3.1: The synthesis of 2,3-diphenylcyclopropenone (**9a** and **9b**)..... 62

Scheme 3.2: The synthesis of pyridinium salts (**10a–g and 11a–g**)..... 62

Scheme 3.3: The synthesis of pyridinium salts (**10j–m and 11j–m**)..... 63

Scheme 3.4: The synthesis of 6-alkyl, alkoxy or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**14a–v**)..... 63

CHAPTER 4.0

Scheme 4.1: The synthesis of 1,3-diaryl-prop-2-yn-1-ones (**9a–r**)..... 87

Scheme 4.2: The synthesis of substituted-3,4,6-triphenylpyran-2-ones (**11a–s**)..... 87

Scheme 4.3: The synthesis of substituted-3,4,6-triphenylpyran-2-ones (**12a–s**)..... 88

Scheme 4.4: The synthesis of substituted-3,4,6-triphenylpyran-2-ones (**12t** and **12u**)..... 88

Scheme 4.5: The synthesis of substituted-3,4,6-triphenylpyran-2-ones (**12v** and **12w**)..... 89

Scheme 4.6: The synthesis of substituted-3,4,6-triphenylpyran-2-ones (**12x**)..... 89

Scheme 4.7: The synthesis of substituted-3,4,6-triphenylpyran-2-ones (**12y**)..... 90

Scheme 4.8: The synthesis of substituted-3,4,6-triphenylpyran-2-ones (**13a** and **13b**)..... 90

Scheme 4.9: The synthesis of substituted-3,4,6-triphenylpyran-2-ones (**13c–f**)..... 91

Scheme 4.10: The synthesis of substituted-3,4,6-triphenylpyran-2-ones (**17a–c**)..... 91

GENERAL DISCUSSION AND CONCLUSIONS

Scheme 6.1: The synthesis of 6-alkyl, alkoxy or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**5**)..... 137

Scheme 6.2: The synthesis of substituted-3,4,6-triphenylpyran-2-ones (**9**)..... 140

LIST OF FIGURES

INTRODUCTION

Figure 1.0.1: Biosynthetic pathway for the formation of naturally occurring prostanoids..... 1

Figure 1.0.2: A. Ribbon diagram of the ovine COX-1 homodimer with flurbiprofen bound within the COX active site. B. Ribbon diagram of ovine COX-1 monomer with flurbiprofen bound, indicating the locations of the COX and peroxidase (POX) active sites and the EGF and MBD domains..... 3

Figure 1.0.3: A schematic representation of the branched chain reaction mechanism for the COX enzymes..... 3

Figure 1.0.4: A schematic representation of the conversion of AA to prostaglandins PGG_2 and PGH_2 respectively..... 4

Figure 1.0.5: A. Active site of COX-1. B. Active site of COX-2..... 6

Figure 1.0.6: NSAID binding in the COX active site with flurbiprofen (yellow) bound in the ovine COX-1 active site channel..... 17

Figure 1.0.7: The ribbon diagram of the murine COX-2 enzyme with the diaryheterocyclic selective COX-2 inhibitor SC-558 (represented as space filling model) bound to the COX-2 active site..... 18

Figure 1.0.8: The diaryheterocyclic selective COX-2 inhibitor SC-558 (yellow colored) bound to the murine COX-2 active site..... 19

Figure 1.0.9: Overlay of the diaryheterocyclic selective COX-2 inhibitor SC-558 (yellow) with indomethacin analog (**55**) in green docked into the murine COX-2 active site. The sulfone moiety of SC-558 and **55** are in the same plane within the COX-2 secondary pocket..... 20

CHAPTER 1.0

Figure 1.1: Docking of (*E*)-1,1-dihalo-2-(4-methanesulfonylphenyl)-3-phenylcyclopropane (**11h**) on the active site of murine COX-2.. 39

Figure 1.2: Docking of (*Z*)-1,1-dihalo-2-(4-methanesulfonylphenyl)-3-phenylcyclopropane (**10h**) on the active site of murine COX-2... 40

CHAPTER 2.0

Figure 2.1: Docking of **12b** (ball and stick) in the active site of murine COX-2 (line and stick)..... 56

CHAPTER 3.0

Figure 3.1: Docking of 6-ethoxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (**14i**) in the active site of murine COX-2..... 66

Figure 3.2: Docking of 6-ethoxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (**14i**) in the active site of ovine COX-1..... 67

CHAPTER 4.0

Figure 4.1: Docking 6-(4-methoxyphenyl)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (**12e**) (ball and stick) in the active site of murine

COX-2..... 94

Figure 4.2: Docking 6-(4-methoxyphenyl)-4-(4-methanesulfonylphenyl) -3-phenylpyran-2-one (**12o**) (ball and stick) in the active site of murine COX-2..... 95

Figure 4.3: Overlay of the binding modes of **12e** and **12o** within the active site of murine COX-2..... 97

Figure 4.4: Docking 6-(4-methoxyphenyl)-3-(4-azidophenyl)-4-phenyl pyran-2-one (**17a**) (ball and stick) in the active site of murine COX-2..... 98

GENERAL DISCUSSION AND CONCLUSIONS

Figure 6.1: Docking of the rofecoxib analog (**19b**, ball and stick) in the active site of murine COX-2..... 144

Figure 6.2: Docking of the celecoxib analog (**21a**, ball and stick) in the active site of murine COX-2..... 145

Figure 6.3: Docking of the celecoxib analog (**21b**, ball and stick) in the active site of murine COX-2..... 146

LIST OF TABLES

1.0.0.0. INTRODUCTION

Table 1.0.1: Comparison of COX-1 and COX-2 properties..... 5

Table 1.0.2: Classification of NSAIDs according to their COX-1/2 inhibitory activities..... 9

2.0.0.0. CHAPTER 1.0

Table 1.1: Antiinflammatory and analgesic activities, molecular volumes and cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) Inhibition activities for (*Z*)- and (*E*)-1,1-dihalocyclopropanes... 38

3.0.0.0. CHAPTER 2.0

Table 2.1: In vitro inhibition of COX-1 and COX-2 by 6-alkyl, 6-alkoxy or 6-alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**10a–c**, **11a–c**, and **12a–c**)..... 55

4.0.0.0. CHAPTER 3.0

Table 3.1: COX-1/COX-2 inhibitory and antiinflammatory-analgesic activities of 6-alkyl-, 6-alkoxy-, and 6-alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**14a–v**)..... 64

5.0.0.0. CHAPTER 4.0

Table 4.1: COX-1/COX-2 inhibitory activities of substituted-3,4,6-triphenylpyran-2-ones (**12a–y**)..... 92

Table 4.2: COX-1/COX-2 inhibitory activities of substituted-3,4,6-triphenylpyran-2-ones (**13a–f** and **17a–c**)..... 93

Table 4.3: Antiinflammatory and analgesic activities of substituted-3,4,6-triphenylpyran-2-ones (**12e–f**, **12t**, **13f** and **17a**)..... 99

6.0.0.0. GENERAL DISCUSSION AND CONCLUSIONS

Table 6.1: In vitro inhibition of COX-1 and COX-2 by 3-(2-, 3- and 4-acetoxyphenyl) analogs of rofecoxib (**19a–c**)..... 143

LIST OF CHARTS

CHAPTER 1.0

Chart 1.1: The structures of some selective cyclooxygenase-2 (COX-2) inhibitors having a central 4-, 5- or 6-membered ring system... 35

CHAPTER 2.0

Chart 2.1: Representative examples of diarylheterocyclic selective COX-2 inhibitors..... 53

CHAPTER 3.0

Chart 3.1: Representative examples of selective tricyclic COX-2 inhibitors..... 61

CHAPTER 4.0

Chart 4.1: Representative examples of tricyclic selective COX-2 inhibitors..... 86

LIST OF APPENDICES

CHAPTER 1.0

Appendix 1.1: Elemental analysis for compounds **8e, 10d, 10h-l, 11c, 11d** and **11e–l**..... 52

CHAPTER 3.0

Appendix 3.1: Elemental analysis for compounds **11j** and **14a–v**..... 84

CHAPTER 4.0

Appendix 4.1: Elemental analysis for compounds **8, 9, 11, 12, 13** and **17**..... 130

1.0.0.0. INTRODUCTION

Prostaglandin H synthase (PGHS), also known as cyclooxygenase (COX), is a rate-limiting enzyme in the production of prostaglandins and thromboxanes, that are derived from arachidonic acid (AA). The most significant step implicating prostaglandins in inflammation was the discovery by Vane in 1971 that aspirin, a well known nonsteroidal antiinflammatory drug (NSAID), is an inhibitor of cyclooxygenase.[1] Prostaglandins are mediators of inflammation primarily due to their involvement in the regulation of vascular tone and tissue permeability. The induction of inflammation has been extensively studied and is very complex. Prostanoids have long been known to behave as important physiological and pathological mediators that have been implicated in a number of therapeutic areas of interest including inflammation, pain, pyrexia, cancer, glaucoma, male sexual dysfunction, osteoporosis, cardiovascular disease, labor and asthma.[2]

In the early 1990s, an inducible isoform of COX was identified as distinct from the constitutive enzyme which led to the recognition that two different cyclooxygenases exist, namely COX-1 and COX-2. The observations that expression of COX-2 can be upregulated by inflammatory stimuli and that COX-1 was expressed constitutively in most

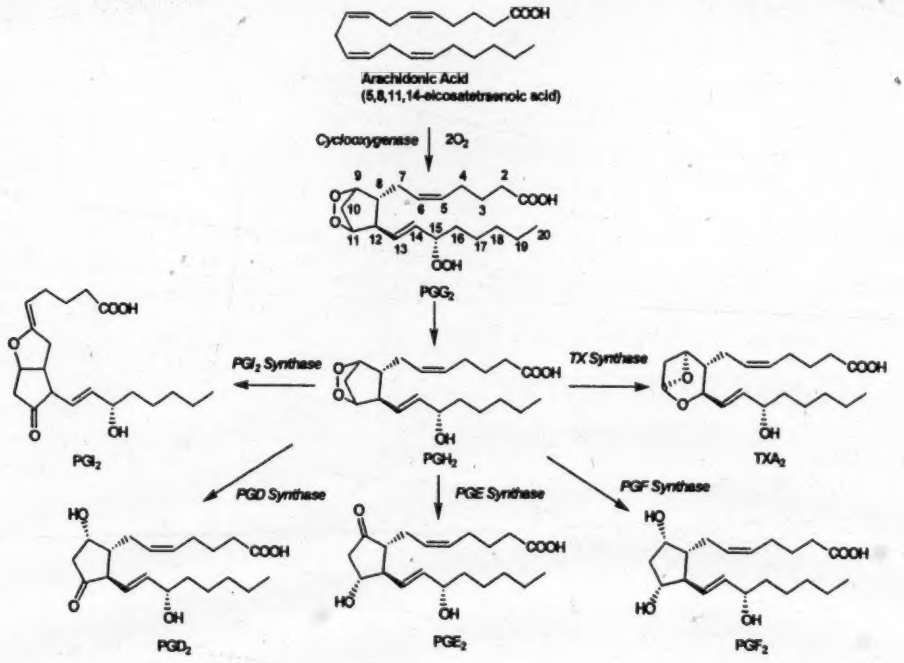


Figure 1.0.1: Biosynthetic pathway for the formation of naturally occurring prostanoids.

tissues led to the hypothesis for the existence of 'good' versus 'bad' COX with COX-1 producing prostaglandins required for normal physiological functions. In contrast, prostaglandin synthesis induced by COX-2 is responsible for inflammatory reactions.[3] This theory led to the development of antiinflammatory-analgesic agents which inhibit COX-2 selectively and provide drugs with a reduced gastrointestinal (GI) toxicity profile. Currently selective COX-2 inhibitors such as Celebrex® and Vioxx® are marketed for the treatment of inflammatory conditions such as rheumatoid arthritis (RA) and osteoarthritis (OA). In addition, the COX-2 enzyme is currently an attractive therapeutic target in the treatment of other disease states that include tissue-specific cancers and neurodegenerative disorders.[4]

1.1.0.0. THE CYCLOOXYGENASES

The two known COX isoforms share a high degree of amino acid sequence similarity and are bifunctional enzymes. They catalyse the first committed step in the biosynthesis of prostaglandins, thromboxanes and other eicosanoids. The production of these eicosanoids is regulated by the availability of AA and the release of AA from membrane phospholipid is mediated by phospholipases. Once AA is released, the COX isoforms catalyze two sequential reactions. The initial cyclooxygenase reaction converts AA to prostaglandin G_2 (PGG_2). The subsequent peroxidase reaction reduces PGG_2 to prostaglandin H_2 (PGH_2). The cell specific isomerization or reduction of PGH_2 produces biologically active end products such as prostacyclin (PGI_2), prostaglandin E_2 (PGE_2), prostaglandin D_2 (PGD_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and thromboxanes (TXA_2) respectively as shown in Figure 1.0.1.[5]

1.2.0.0. THE CYCLOOXYGENASE STRUCTURE

The COX isoforms are heme containing enzymes that exhibit distinctly different expression profiles and roles in several physiological processes. The primary structure of COX-1 is comprised of 602 amino acid residues whereas COX-2 contains 604 amino acid residues. The residues of COX are numbered by convention to the ovine or murine COX-1 sequence to standardize structural and functional comparisons between species.[6] The COX isoforms share 60-65% sequence identity within species and about 85-90% sequence identity among different species. The first three-dimensional structure of a COX enzyme, the ovine COX-1 complexed with the NSAID flurbiprofen was published by Picot et al. in 1994.[7] The structures of human and murine COX-2 are virtually superimposable on the ovine COX-1. The COX isoforms are homodimers both functionally and structurally. The general feature of each monomer is the presence of three structural domains; a *N*-terminal epidermal growth factor (EGF) domain possessing 50 amino acids, a neighbouring membrane binding domain (MBD) having about 50 amino acids, and a large catalytic domain made up of about 460 amino acids as shown in Figure 1.0.2. The MBDs of COX are made up of four short, consecutive, amphipathic α helices, with helix D merging into the catalytic

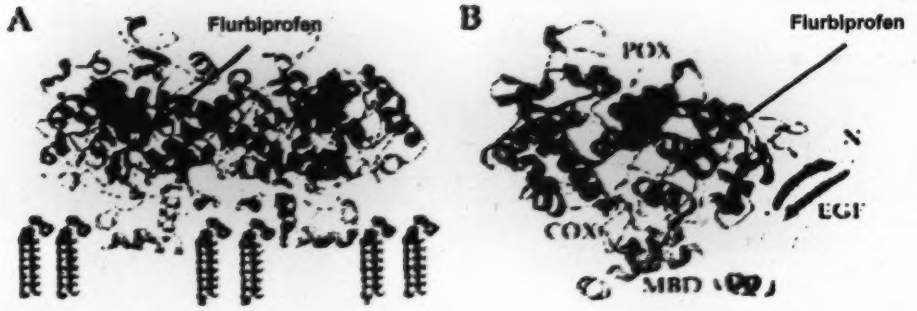


Figure 1.0.2: A. Ribbon diagram of the ovine COX-1 homodimer with flurbiprofen bound within the COX active site. B. Ribbon diagram of ovine COX-1 monomer with flurbiprofen bound, indicating the locations of the COX and peroxidase (POX) active sites and the EGF and MBD domains (adapted from ref 6).

domain. These helices surround an opening through which fatty acid substrates and NSAIDs are believed to enter the COX active site. The upper half of the catalytic domain is the COX active site that binds fatty acid substrates and NSAIDs. Both COX-1 and -2 are attached to endoplasmic reticulum (ER) and nuclear envelope through the C-terminal sequences and are present on the luminal surfaces of ER and inner and outer membranes of the nuclear envelope. *N*-glycosylation of COX isoforms is required for enzyme folding and activity.

1.2.1.0. Cyclooxygenase (COX) and peroxidase (POX) activities

Both COX isoforms catalyze a cyclooxygenase reaction in which the substrate AA and two molecules of molecular O_2 are converted to PGG_2, and a peroxidase reaction in which PGG_2 is reduced to PGH_2 by a two electron reduction. These two reactions occur at distinct but structurally and functionally interconnected sites as shown in Figure 1.0.2. Both cyclooxygenase and peroxidase activities require heme as a cofactor.

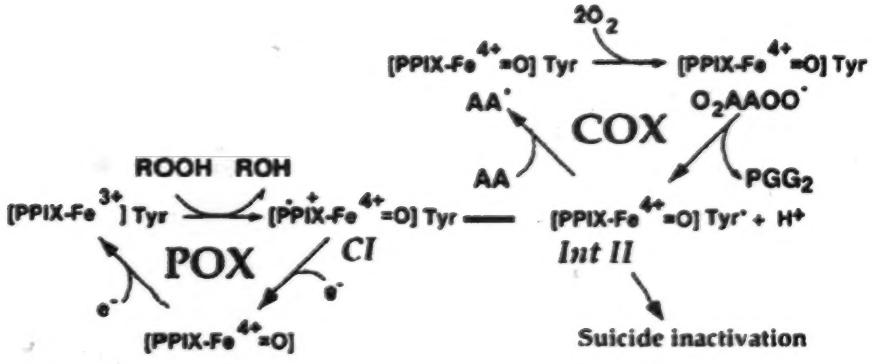


Figure 1.0.3: A schematic representation of the branched chain reaction mechanism for the COX enzymes (adapted from ref 6).

The cyclooxygenase reaction occurs in a hydrophobic channel in the core of the enzyme whereas the peroxidase site is in the heme containing area near the protein surface.[6] Interestingly, peroxidase can operate independently of cyclooxygenase, whereas the cyclooxygenase activity is dependent on peroxidase activity that requires the oxidation of the heme group.[8]

A branched chain mechanistic model was first proposed by Ruf and coworkers as shown in Figure 1.0.3.[8] According to this scheme, heme iron reacts with a hydroperoxide substrate thereby initiating a two-electron oxidation yielding compound I (CI), which corresponds to an enzyme state with an oxyferryl heme radical cation. The rapid single electron reduction of compound I via an intramolecular migration of the radical from the heme group to the amino acid residue Tyr^{385} at the top of COX active site creates intermediate II (Int II).[9] The tyrosyl radical thus formed initiate, the COX reaction by abstracting the 13-pro-*S* hydrogen from the COX substrate AA, thereby forming the arachidonyl radical.[10] This fatty acid radical reacts with molecular O_2 to produce an 11-hydroperoxyl radical, which forms the endoperoxide cyclopentane moiety of PGG_2, whereas the addition of a second O_2 molecule at carbon 15 produces PGH_2 as shown in Figure 1.0.4.[11, 12]

Both COX and POX activities undergo suicide inactivation during catalysis and their activities fall to zero within 1-2 min even in the presence of sufficient substrates. Although the exact mechanisms for suicide inactivation is not yet resolved, it is likely that it proceeds from the Intermediate II.[13]

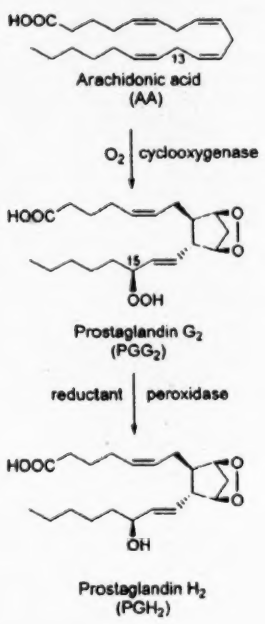


Figure 1.0.4: A schematic representation of the conversion of AA to prostaglandins PGG_2 and PGH_2 respectively (adapted from ref 11).

Recently, on the basis of the structure of PGH_2 bound at the COX active site, a working model for the mechanistically competent conformation of AA that leads to prostaglandin biosynthesis has been proposed.[14]

1.2.1.1. Comparisons of COX-1 and COX-2

A second isoform of COX-2 was independently discovered in the late 90's by different groups.[15,16] COX-2 expression is inducible by a wide range of extracellular and intracellular stimuli such as lipopolysaccharide (LPS), interleukin-1 (IL-1), and tumor necrosis factor alpha (TNF-α) whereas COX-1 is expressed constitutively in most tissues.

Table 1.0.1: Comparison of COX-1 and COX-2 properties.

Parameters	COX-1	COX-2
Gene size	22 kb	8.3 kb
Exons	11	10
Chromosome	9q32-q33.3	1q25.2-q25.3
mRNA	2.8 kb	4.1 kb
mRNA regulation	constitutive	inducible
Inducers	———	LPS, cytokines, phorbol esters
Molecular weight	70 kD	70-72 kD
Localization	Nuclear membrane, ER	Nuclear membrane, ER
Cofactors	1 mol of heme	1 mol of heme
Glycosylation	-N, 3 sites	-N, 3 or 4 sites
Substrate specificity	AA, γ-linolenic acid	AA, γ-linolenic acid, α-linolenic acid, Eicosapentenoic acid
Activity	23 mmol AA/mg/min	11 mmol AA/mg/min

The genomic structure of both human and murine COX-1 is composed of 11 exons and 10 introns spanning 22.5 kb. The human COX-1 maps to chromosome 9q32-q33.3. By comparison, the human COX-2 gene is only 8.3 kb in size and is localized to chromosome 1q25.2-q25.3.[17-19] Both COX-1 and COX-2 have very similar cyclooxygenase active site structures, catalytic mechanisms and products. Table 1.0.1 shows some of the differences between human COX-1 and COX-2.

1.2.1.2. Structural differences between COX-1 and COX-2

The COX-1 and -2 monomers each contain a 25 Å hydrophobic channel that originates at the membrane binding domain (MBD) and extends into the core of the catalytic domain. The MBD forms the mouth and first half of the channel, allowing AA and O_2 to enter directly from the apolar compartment of the lipid layer. The three dimensional X-ray crystal structures of either murine or human COX-2 can be superimposed on that of COX-1.[7, 20-22] Within the catalytic sites of COX-1 and –2, one critical structural difference occurs at position 523.

At the amino acid residue position 523, COX-1 has a bulkier isoleucine (Ile) whereas the smaller valine (Val) is present in COX-2. This minor change makes the overall molecular volume of the COX-2 active site almost 20% larger than that of COX-1. Consequently, an additional secondary pocket is accessible in the COX-2 active site as shown in

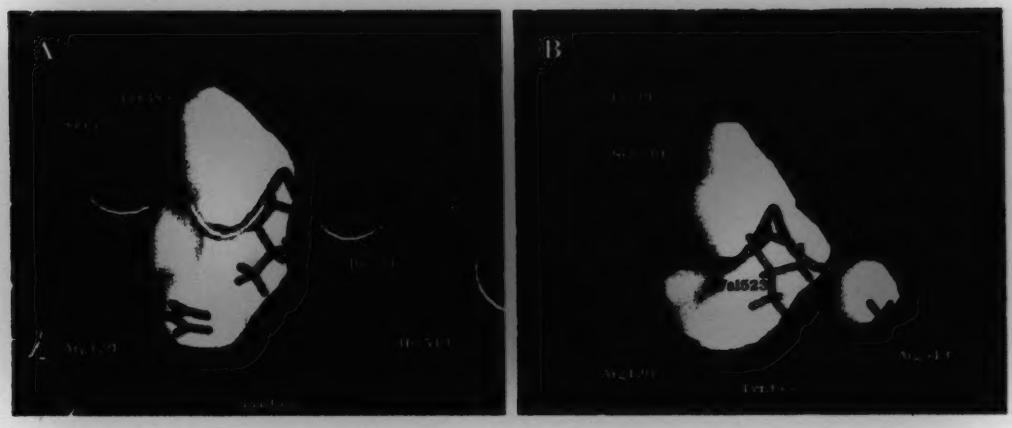


Figure 1.0.5: A. Active site of COX-1. B. Active site of COX-2 (adapted from ref 20).

Figure 1.0.5. [20] This structural difference has been exploited in designing selective COX-2 inhibitors. The carboxylic acid moiety of the COX substrate AA and nonselective NSAIDs undergo electrostatic interaction with Arg^{120} which is not possible with diarylheterocyclic selective COX-2 inhibitors.[21,23,24] Other structural differences exist at amino acid residues 434 and 513 (numbering scheme based on ovine COX-1). The COX-1 isoform has Ile at 434 whereas COX-2 has Val. Similarly, at position 513, COX-1 has histidine (His) and COX-2 has arginine (Arg). These subtle differences provide more substrate flexibility in the COX-2 active site.

1.2.1.3. Regulation and expression of COX-1 and COX-2

The COX-1 isoform is constitutively expressed at high levels in cells and tissues such as endothelium, monocytes, platelets, renal collecting tubules and seminal vesicles, indicating that it is developmentally regulated.[25] Targeted gene disruption has been well, and have no gastric pathology and show less indomethacin induced gastric ulceration than COX-1 wild type mice. COX-1 null mice also exhibit reduced platelet aggregation and decreased inflammatory response.[26, 27]

The COX-2 enzyme is induced by mediators of inflammation such as LPS, IL-1, TNF-α in a wide variety of cells and tissues such as vascular endothelium, osteoclasts, rheumatoid synovial endothelial cells, monocytes and macrophages. Recent studies have also shown that constitutively expressed COX-2 plays specific functions in reproduction, renal physiology, bone resorption and neurotransmission.[28-31]

1.2.1.4. Role of COX enzymes in inflammation

Traditional NSAIDs prescribed to control joint pain to treat inflammatory conditions such as RA and OA produce their antiinflammatory and analgesic properties by nonselective inhibition of COX activity. During the inflammatory process, the COX-1 mRNA and protein activity do not change whereas a dramatic increase in COX-2 levels is seen,

leading to increased production of proinflammatory PGs. The GI side effects associated with traditional NSAIDs are due to the inhibition of gastroprotective PGs synthesized via the COX-1 pathway.[32, 33] The expression of COX-2 has been extensively studied in animal models of inflammation and there is strong evidence that induction of the COX-2 enzyme is associated with inflammation. The COX-1 enzyme appears to be unaffected by the inflammatory process, and similar levels of mRNA and protein are detectable in both normal and inflamed tissue in animal models. At the site of inflammation, local reddening, heat generation, swelling and pain are classic signs of which the former three are caused by increased blood flow and vascular permeability with resultant edema. PGs such as PGE_2 and PGI_2 produced via the COX-2 pathway magnify the degree of inflammation initiated by other mediators of inflammation such as histamine and bradykinin, thereby increasing vascular permeability and edema[34-37] COX-2 is not detectable in normal tissue, but is detectable after an inflammatory stimuli. Selective COX-2 inhibitors have shown good antiinflammatory and analgesic activities in different animal models. Clinical trials indicate a reduced GI toxicity profile which confirms the role of the COX-2 enzyme in the inflammatory process.[38-40]

1.2.1.5. COX enzymes in the gastrointestinal system

In humans and several other species, it has been shown that COX-1, but not COX-2, is expressed constitutively throughout the GI system.[41] PGs such as PGE_2 and PGI_2 formed by COX-1 have cytoprotective effects on the GI mucosa as they reduce gastric acid secretion from parietal cells in the stomach, increase mucosal blood flow, and stimulate release of viscous mucus. Selective COX-2 inhibitors are efficient and often superior antiinflammatory agents with less GI toxicity due to their selective inhibition of COX-2 and sparing action on COX-1. However, there are reports of constitutive expression of COX-2 in healthy human and rabbit GI mucosa.[42] It has also been reported that during the GI ulcer formation process, COX-2 may be induced and that it could play a role in GI healing.[43] However, there is little doubt that COX-1 derived PGs are important for the maintenance of GI mucosal integrity.

1.2.1.6. COX enzymes in the kidney

PGs regulate vascular tone and normal blood flow, thereby maintaining renal function.[4] Studies using animal models of renal diseases, and patients with congestive heart failure, liver cirrhosis or renal insufficiency have shown that PGE_2 was primarily responsible for maintaining normal kidney function.[22] In humans, COX-1 is constitutively expressed in the vasculature, the collecting ducts and the loop of Henle, whereas low levels of COX-2 are expressed constitutively in the macula densa, epithelial cells lining the ascending loop of Henle and medullary interstitial cells of the renal papillae.[44] The COX-2 enzyme is involved in normal renal development, and COX-2 deficient mice develop severe nephropathy.[26,45] Studies have shown that NSAID-induced sodium retention in healthy and elderly patients is mediated by the inhibition of COX-2, whereas

decreased glomerular filtration rate is associated with inhibition of COX-1.[46] Thus both enzymes are involved in renal physiology. Recent studies with the selective COX-2 inhibitor rofecoxib have also shown an association with the development of nephritis.[47]

1.2.1.7. COX enzymes and the cardiovascular system

It is known that the COX-1 isozyme is constitutively expressed in platelets and is responsible for the formation of pro-aggregatory TXA_2. In contrast, the synthesis of anti-aggregatory PGI_2 in endothelial cells is catalyzed primarily by COX-2.[48] Aspirin acts as an irreversible inhibitor of the enzyme COX-1 in platelets by acetylating the Ser^{530} residue. This leads to blocking of TXA_2 synthesis, leading to a reduced risk of thrombosis. The COX mediated vascular control has been demonstrated in COX-1 and -2 knock out animal models. Mice deficient in COX-2 die within 48 hours after birth with a patent ductus arteriosus (an arterial connection that directs blood flow away from the pulmonary circulation in fetal life that must close at birth). Similarly mice deficient in both isoforms of COX die within 12 hours of birth with a similar condition.[49]

A special communication has raised a cautionary flag regarding the use of COX-2 inhibitors in patients at risk for cardiovascular morbidity such as myocardial infarction that has been explained using the following facts.[50] PGI_2 is a vasodilator and a potent inhibitor of platelet aggregation produced by COX-2 at the sites of inflammation. Although selective COX-2 inhibitors have no effect on TXA_2 production, by decreasing PGI_2 production, selective COX-2 inhibitors may tip the natural balance between prothrombotic TXA_2 and antithrombotic PGI_2 that could potentially increase the possibility of a thrombotic cardiovascular event. There are other indications of a protective role for PGE_2 and PGI_2 derived from the COX-2 pathway pertaining to oxidative damage.[51] Therefore, both COX-1 and -2 derived PGs appear to have a profound role in the regulation of vascular homeostasis.

1.3.0.0. SELECTIVE CYCLOOXYGENASE-2 (COX-2) INHIBITORS

NSAIDs (nonsteroidal antiinflammatory drugs) are the most widely used drugs for the treatment of joint inflammation and musculoskeletal disorders. In the US alone, more than 15 million individuals use NSAIDS daily. Since the discovery of the COX-2 isozyme, numerous studies have shown that selective COX-2 inhibition produces effective antiinflammatory and analgesic activity whereas COX-1 inhibition is associated with side effect profiles exhibited by traditional NSAIDs. GI toxicity associated with NSAID therapy is responsible for 200,000-400,000 hospitalizations each year in the US alone at a cost of $0.8-1.6 billion.[52] Current research is focused on developing safer NSAIDs with increased COX-2 selectivity and potency. The COX-2 selectivity (Selectivity Index; SI) of traditional NSAIDs have been tested using various assay systems, including purified recombinant enzyme, transfected cells, and whole blood assays. The SI is expressed as the ratio of the COX-2 IC_{50} to the COX-1 IC_{50} value (Table 1.0.2)[53, 54]

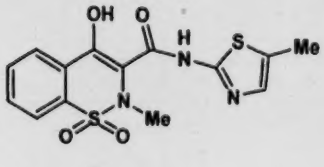
Table 1.0.2: Classification of NSAIDs according to their COX-1/2 inhibitory activities (adapted from ref 54).

Class	Properties	Examples
Group 1	NSAIDs that can inhibit both COX-1 and COX-2 completely with little selectivity	Aspirin, diclofenac, flurbiprofen, indomethacin, piroxicam
Group 2	NSAIDs that inhibit COX-2 with a 5-50 fold selectivity	Celecoxib, etodolac, meloxicam, nimesulide
Group 3	NSAIDs that inhibit COX-2 with a > 50 fold selectivity	Rofecoxib, etoricoxib
Group 4	NSAIDs that are weak inhibitors of both isoforms	5-aminosalicylic acid, sodium salicylate, nabumetone, sulfasalazine

The differential tissue distribution of the COX-1 and -2 enzymes has made it possible to predict the therapeutic and toxicity profiles of selective COX-2 inhibitors based on their COX-1/2 inhibitory profiles. For example, in the treatment of arthritis, by obtaining data from concentration/time data of COX-2 inhibitors in the synovial/systemic compartments (pharmacokinetic profile) and the selectivity for COX-2/COX-1 (pharmacodynamic profile), one can predict antiinflammatory effects versus toxicity profiles.[55]

Traditional NSAIDs such as meloxicam (**1**), nimesulide (**2**) and etodolac (**3**) were not designed specifically as COX-2 inhibitors, but were identified from pharmacological tests as potent antiinflammatory agents with low GI toxicity profiles in animal models prior to the discovery that there are two COX isozymes. These drugs are now known to preferentially inhibit COX-2 rather than COX-1.[56-58]

In recent years extensive research has been directed toward the development of selective COX-2



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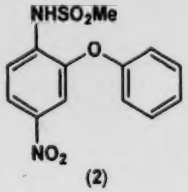

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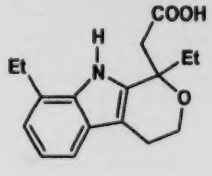


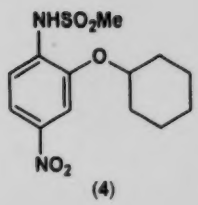
(3)

inhibitors. Successful marketing of selective COX-2 inhibitors have reinforced their usefulness to treat inflammatory conditions. Now, a league of the top 20 drugs includes the selective COX-2 inhibitors celecoxib (Celebrex®) and rofecoxib (Vioxx®) and together, they represented global sales in excess of $450 million US in the year 2001.[59] In addition, other potential applications of selective COX-2 inhibitors for the treatment of a wide range of other disease states is being investigated.[4]

1.3.1.0. CHEMICAL CLASSIFICATION OF SELECTIVE COX-2 INHIBITORS

1.3.1.1. Methanesulfonanilide inhibitors

Members of the methanesulfonanilide class of COX-2 inhibitors generally exhibit preferential COX-2 selectivity (COX-2 selectivity anywhere between 5-50, Table 1.0.2). These compounds are characterized as derivatives of alkylsulfonanilides. Nimesulide (**2**) was the first member of this class to be discovered. Pharmacological studies have demonstrated nimesulide's clinical anti-inflammatory properties.[60] Structural modification of nimesulide led to the development of NS-398 (**4**) with preferential COX-2 selectivity and antiinflammatory activities.[61] Incorporation of an electron withdrawing substituent into the five-membered carbocyclic ring in L-745,337 (**5**) increased COX-2 inhibitory potency and selectivity.[62]



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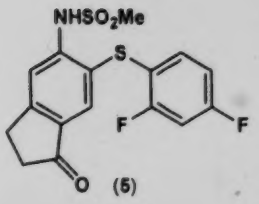

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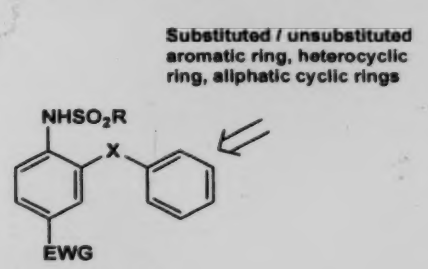
The general structural features present in the methanesulfonanilide class of COX-2 inhibitors are shown below.

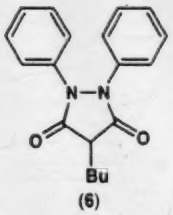


1.3.1.2. Diarylheterocycles as selective COX-2 inhibitors

Currently, diarylheterocycles constitute a major class of selective COX-2 inhibitors. The historical origins of diarylheterocycles as pharmacophores can be traced back to the antiinflammatory agent phenylbutazone (**6**) which stimulated medicinal chemists worldwide to further explore diarylheterocycles. In this regard, researchers at DuPont initiated an extensive program in the 1970's to evaluate novel diarylheterocycles as antiinflammatory agents, which led to the discovery of a very potent and selective COX-2 inhibitor DuP-697 (**7**) possessing a central 5-membered thiophene ring.

A common structural feature of these tricyclic molecules is the presence of 1,2-diaryl substitution on a central 4-,

5- or 6-membered ring system. Structure-activity relationship (SAR) studies have



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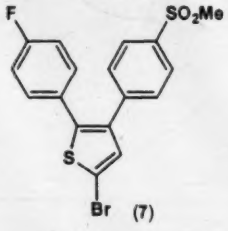
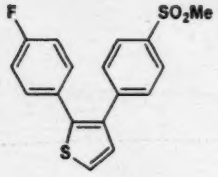


(7)

shown that for optimum COX-2 selectivity and inhibitory potency a -SO_2Me, or a -SO_2NH_2 substituent at the *para*-position of a phenyl ring was essential, and that the presence of a *para*-F-substituent on the non-sulfonyl vicinal phenyl ring often improves in vivo activity.[60]

1.3.1.3. Diarylheterocycles with a central 5-membered thiophene ring



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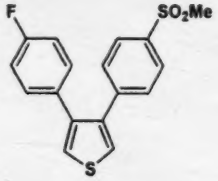

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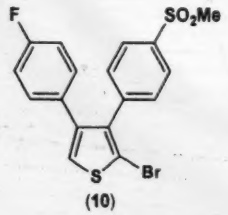
The 2,3-diphenylthiophene DuP-697 (**7**) exhibited excellent COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.01 μM, COX-1 IC_{50} = 1 μM; S.I. = 100).[60] However, this drug failed in the clinic due to an unusually long plasma half life. Replacement of the bromine atom on the central thiophene provided compounds with better pharmacokinetic profiles.[63] Generally, for this class of diarylheterocycles, the presence of a *p*-SO_2NH_2 moiety resulted in increased COX-2 inhibitory potency and improved oral absorption.[64,65]

The regioisomeric 3,4-diarylthiophenes were found to be COX-2 selective. Compounds **8** and **9** exhibited similar activity profiles (COX-2 IC_{50} = 0.08 μM, COX-1 IC_{50} > 100 μM), whereas the two mono bromo regioisomers (**10** and **11**) possessed different activity profiles.



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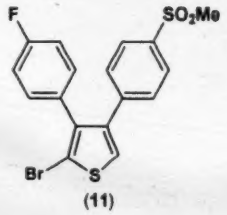

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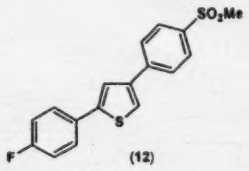
Compound **10** exhibited no inhibition of either enzyme at a concentration of 100 μM whereas **11** was COX-2 selective (COX-2 IC_{50} = 0.08 μM, COX-1 IC_{50} > 100 μM). Compounds **12** and **13** exhibited significantly lower activity profiles thereby showing the importance of vicinal-diaryl substitution for this class of compounds.[66]



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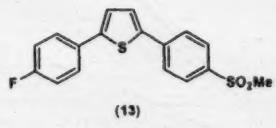

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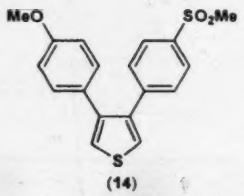
The substitution of the second aryl ring not having the SO_2Me substituent enhanced inhibition of COX-1 in the case of 3,4-diarylthiophenes. For example, the presence of a methoxy group at the *para*-position of **14** showed a COX-1 IC_{50} = 0.9 μM and a COX-2 IC_{50} = 0.08 μM, whereas introduction of a fluorine substituent (**15**) resulted in a further improvement in COX-2

selectivity (COX-2 IC_{50} = 0.03 μM, COX-1 IC_{50} > 100 μM).



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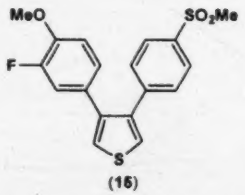

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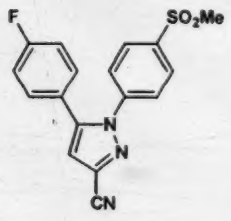
Thus, subtle variations in the structures of diphenylthiophenes can alter COX-2 inhibitory potency and selectivity. In addition, the physicochemical properties can be altered by various structural modifications.

1.3.1.4. Diarylheterocycles with a central 5-membered pyrazole ring

The 1,5-diarylpyrazole class of compounds proved to be a fertile source for highly potent and selective COX-2 inhibitors. The first compound in this series (**16**) exhibited excellent in vitro COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.24 μM, COX-1 IC_{50} > 100 μM) with potent antiinflammatory activity in animal models with no tendency to cause GI damage.[60] In the initial stages of the development of the 1,5-diarylpyrazole class, SC-58125 (**17**) was one of the most extensively characterized compounds.[36] Compound **17** had a very long in vivo half life of > 200 h in the animal models making it unacceptable for clinical use.



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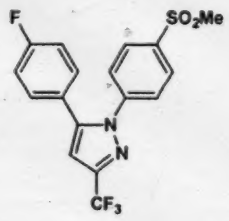
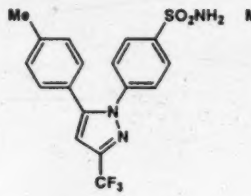


(17)

Replacement of the *para*-SO_2Me group by a SO_2NH_2 substituent provided a significant improvement in the pharmacological profile. Compounds of this type exhibited superior pharmacological and oral bioavailability relative to their methylsulfone counterparts. Extensive studies of this class of compounds led to the successful development of the potent and selective COX-2 inhibitor SC-58635 (**18**) (in vitro COX-2 IC_{50} = 0.04 μM; COX-1 IC_{50} = 13 μM) with potent in vivo antiinflammatory activity. Compound **18** was selected for clinical evaluation and is now successfully marketed as celecoxib (Celebrex®), the first diarylheterocyclic selective COX-2 inhibitor to enter the market.[67]



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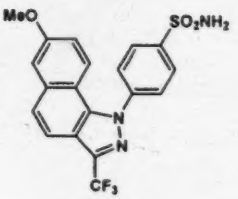

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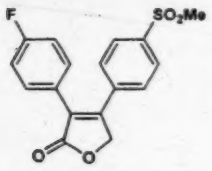
Introduction of conformational strain by an additional ring as in **19** was shown to retain COX-2 selectivity (COX-2 IC_{50} = 0.04 μM, COX-1 IC_{50} > 100 μM).[68] Several research groups continue to investigate this class of compounds in search of better selective COX-2 inhibitors.[69]

1.3.1.5. Diarylheterocycles with a central 5-membered furanone ring

Extensive evaluation of the 3,4-diarylfuranone class of compounds indicated that compound **20** was a selective COX-2 inhibitor (COX-2 IC_{50} = 0.01 μM, COX-1 IC_{50} > 4.7 μM),

whereas the regioisomer **21** was inactive. However, replacement of the *para*-SO_2Me substituent of **20** by a *para*-SO_2NH_2 substituent (**22**) lead to a decrease in COX-2 selectivity (COX-2 IC_{50} = 0.8 μM, COX-1 IC_{50} = 5.8 μM).[70]



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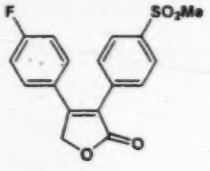

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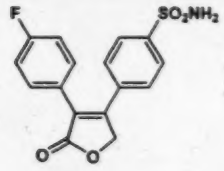
Further lead optimization for this class of compounds by Merck and Co, led to the successful development and marketing of the highly selective and potent COX-2 inhibitor rofecoxib (**23**, Vioxx®). Rofecoxib is an effective antiinflammatory and analgesic agent that does not cause GI toxicity and is a selective in vitro COX-2 inhibitor (IC_{50} = 0.02 μM, COX-1 IC_{50} > 15 μM).[70, 71]



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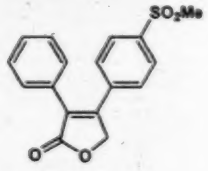
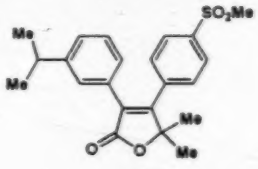


(23)

Other modifications in the diarylfuranone class such as 4,5-diarylfuranones have been reported to possess good in vitro COX-2 inhibitory potency and selectivity with optimal in vivo antiinflammatory properties.[72] For example, the 2,2-dimethyl-4,5-diarylfuranones (**24**, COX-2 IC_{50} = 0.03 μM, COX-1 IC_{50} = 30 μM) and (**25**, COX-2 IC_{50} = 0.05 μM, COX-1 IC_{50} = 50 μM) exhibited excellent in vitro COX-2 inhibitory potency and selectivity. Other variations in the 3,4-diarylfuranone class of compounds include the (*S*)-enantiomer of compound **26** with optimum COX-2 inhibitory and metabolic profile.[73]



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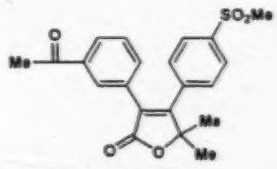
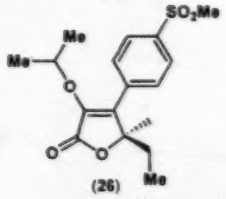


(25)

The oral absorption of compounds belonging to the 3,4-diarylfuranone class can be increased by the introduction of a hydroxyl substituent at the C-5 of the central furanone ring, as in the case of **27**, while retaining COX-2 selectivity (COX-2 IC_{50} = 0.16 μM, COX-1 IC_{50} > 100 μM).[74]



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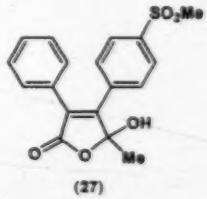
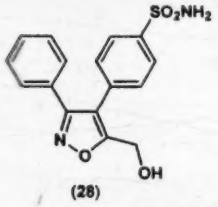


(27)

1.3.1.6. Diarylheterocycles with a central 5-membered isoxazole ring

A large number of regioisomeric diarylisoxazoles have been evaluated as selective COX-2 inhibitors. Scientists at Searle reported that **28** is a potent and selective COX-2 inhibitor (COX-2 IC_{50} = 0.18 μM, COX-1 IC_{50} > 1000 μM) with an excellent in vivo activity profile.[60] Lead optimization of diarylisoxazoles led to the development of a potent and selective COX-2 inhibitor which had a *para*-SO_2NH_2 substituent (**29**, COX-2 IC_{50} = 0.005 μM, COX-1 IC_{50} = 140 μM). The 5-methyl compound **29** is converted

in an in vivo rodent model to its active 5-hydroxymethyl metabolite **28**. Low levels of the metabolite **28** were also found in humans.[75] Currently, compound **29** is marketed as valdecoxib (Bextra®), a second generation selective COX-2 inhibitor with analgesic and antiinflammatory properties.[76] Recently, a water soluble prodrug of valdecoxib (Parecoxib sodium, Dynastat®) was launched as an injectable COX-2 inhibitor possessing antiinflammatory and analgesic activities.[77]



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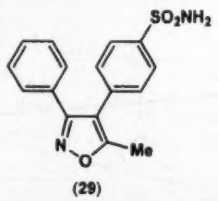
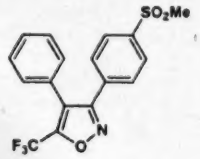


(29)

In this regard, studies in the Knaus research program have shown that the regioisomeric 3,4-diarylisoxazoles with a *para*-SO_2Me substituents exhibit excellent in vitro COX-2 inhibitory activity and in vivo antiinflammatory activities. For example, compound **30** was a potent and highly selective COX-2 inhibitor (COX-2 IC_{50} < 0.005 μM, COX-1 IC_{50} > 500 μM). In contrast, the corresponding regioisomer **31** was a less potent and less selective COX-2 inhibitor (COX-2 IC_{50} = 0.23 μM, COX-1 IC_{50} = 256 μM).[78]



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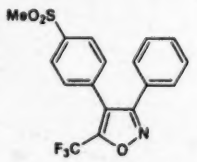
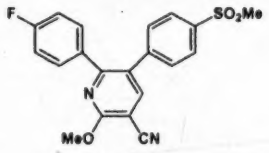


(31)

1.3.1.7. Diarylheterocycles with a central 6-membered pyridine ring

Tricycles with either a 2,3-diarylpyridine or 3,4-diarylpyridine ring system have been investigated as selective COX-2 inhibitors with compounds **32** and **33** exhibiting good in vitro COX-2 selectivity profiles. However, compounds **32** and **33** exhibited poor in vivo antiinflammatory activities.[60,79]



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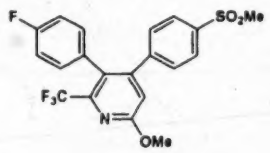
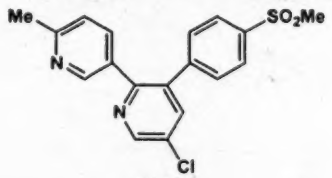


(33)

From this novel class of compounds, Merck and Co successfully developed the orally active potent and selective COX-2 inhibitor etoricoxib (**34**, Arcoxia®) which exhibited clinically acceptable antiinflammatory and analgesic activity with no reports of gastric damage in animal studies and during clinical trials. This second generation selective COX-2 inhibitor showed an in vitro COX-2 IC_{50} = 0.08 μM and COX-1 IC_{50} = 12 μM values.[79-81]

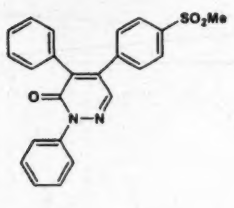


(34)

1.3.1.8. Diarylheterocycles with a central 6-membered pyridazinone ring

Recent studies have shown that the pyridazinone ring can serve as excellent core template for designing selective COX-2 inhibitors. Structure

activity relationship (SAR) studies employing pyridazinones have shown that *N*-substitution is a requirement for COX-2 selectivity as exemplified by **35** (in vitro COX-2 IC_{50} = 0.08 μM; COX-1 IC_{50} > 10 μM). In the *N*-benzyl series, alkoxy substituted compound **36** showed excellent in vitro selective COX-2 inhibition (COX-2 IC_{50} = 0.02 μM; COX-1 IC_{50} > 10 μM) and in vivo activity.[82]



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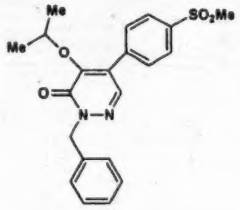


(36)

1.3.1.9. Diarylheterocycles with a central 6-membered pyranone ring

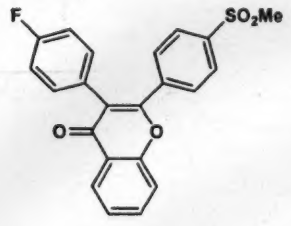
A novel class of 2,3-diarylbenzopyran-4-ones was reported recently that exhibits potent in vitro COX-2 inhibitory potency and selectivity.[83] Replacement of the *para*-SO_2Me substituent in **37** by a *para*-SO_2NH_2 moiety provided compound **38** with increased in vitro COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.06 μM; COX-1 IC_{50} = 67 μM). The introduction of a nitrogen-containing aromatic ring such as pyridine in **39** resulted in a better in vivo activity profile.

1.3.2.0. Diarylcarbocycles as selective COX-2 inhibitors

In the design of selective COX-2 inhibitors various types of tricyclic diarylcarbocycles have been evaluated extensively. Examples include diarylcarbocycles with a central 4-membered cyclobutene or cyclobutenone, a 5-membered cyclopentenes or cyclopetenone, and a 6-membered aromatic ring such as benzene.[60]

1.3.2.1. Diarylcarbocycles with a central 4-membered cyclobutene or cyclobutenone ring

A group of 1,2-diarylcyclobutenes were described as selective COX-2 inhibitors. For this class of compounds, the potency and selectivity was very sensitive to minor steric and electronic changes.[60,84] The 3,4-diarylcyclobutene **40** was a weak inhibitor of COX-2 (COX-2 IC_{50} > 5 μM), but incorporation of a ketone moiety to afford a cyclobutenone central ring (**41**) improved the COX-2 inhibitory activity (COX-2 IC_{50} = 0.11 μM; COX-1 IC_{50} = 2 μM). The COX-2 inhibitory potency was further increased by the incorporation of a geminal dimethyl substituent as in **42** which exhibited potent in vitro COX-2 inhibitory activity (IC_{50} = 0.002 μM; COX-1 IC_{50} = 0.12 μM). On the other hand, the corresponding regioisomer of **42** was a less potent and selective COX-2 inhibitor.



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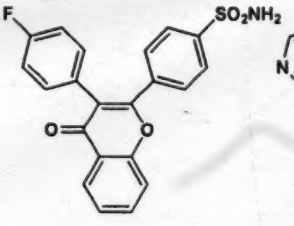

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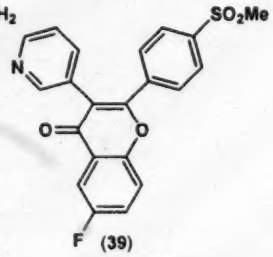

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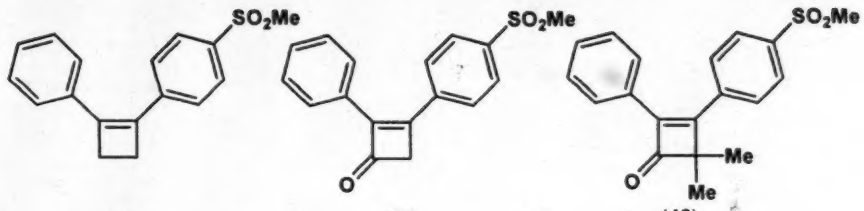
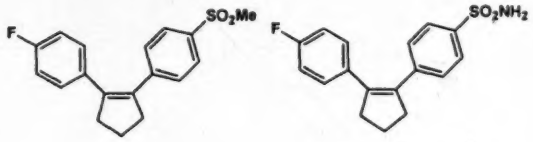


(40) (41) (42)

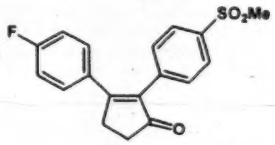
1.3.2.2. Diarylcarbocycles with a central 5-membered cyclopentene or cyclopentenone ring

Diarylcyclopentenes and cyclopentenones were among the first series of compounds to be evaluated for COX-2 selectivity and potency.[60] Searle scientists reported that the diarylcyclopentene SC-57666 (**43**) exhibited a very high degree of in vitro COX-2 inhibitory potency and selectivity. In vivo antiinflammatory activity assays showed **43** was effective and no gastric complications were observed.[85] The replacement of *para*-SO_2Me moiety of SC-57666 by a *para*-SO_2NH_2 moiety as in **44** lead to an improved oral bioavailability.



(43) (44)

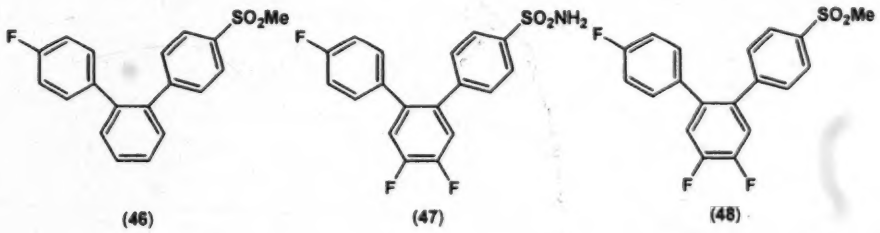
Further modification of **44** by incorporation of a ketone moiety at the 3-position of the central cyclopentene ring afforded **45** with equal COX-2 inhibitory activity and selectivity.[60]



(45)

1.3.2.3. Diarylcarbocycles with a central 6-membered benzene ring system

Novel compounds with a central 6-membered benzene ring template (**46**) were reported to exhibit in vitro COX-2 inhibitory potency and selectivity. Further optimization of **46** by replacement of the *para*-SO_2Me group with a *para*-SO_2NH_2 substituent and additional substitution on the central benzene ring furnished **47** which exhibited excellent in vitro COX-2 inhibitory potency (COX-2 IC_{50} = 0.004 μM; COX-1 IC_{50} = 5.7 μM) and superior in vivo antiinflammatory activity relative to **46**. The corresponding methylsulfone analogue **48** was less potent COX-2 inhibitor (COX-2 IC_{50} = 0.014 μM; COX-1 IC_{50} > 100 μM) but more selective COX-2 inhibitor than **47**.[86]



(46) (47) (48)

1.4.0.0. MOLECULAR MODELING STUDIES OF SELECTIVE CYCLOOXYGENASE-2 (COX-2) INHIBITORS

Following the discovery that inhibition of the inducible isoform of COX produced non-ulcerogenic agents with good antiinflammatory activity, a great deal of research has been focused on the design and development of novel selective COX-2 inhibitors. The first solved X-ray crystal structure was that of the ovine COX-1 isozyme which provided a basic understanding of the 3D structure for this membrane-bound protein.[7] Subsequently, several studies have been published for the X-ray crystal structures of COX-1–ligand complexes.[87,88] In this regard, the most important information was the X-ray crystal structure of the enzyme COX-2 with the diarylheterocyclic selective COX-2 inhibitor SC-558 bound in the active site.[21] This particular enzyme–ligand structure facilitated the rational design of selective COX-2 inhibitors. The *in silico* design of various ligands and their docking into the COX-2 active site has enhanced our understanding of the crucial binding interactions necessary for obtaining COX-2 selectivity.

1.4.1.0. Structural basis for COX inhibition by NSAIDs and selective COX-2 inhibitors

The interactions of NSAIDs within the COX active sites have been studied extensively. All NSAIDs are nonselective inhibitors of both isozymes whereas selective COX-2 inhibitors exhibit tight binding to the COX-2 active site. Traditional NSAIDs exhibit one of three different modes of binding: i) reversible binding (e.g. ibuprofen), ii) rapid, low affinity reversible binding followed by a time-dependent, higher affinity, slowly reversible binding (eg: flurbiprofen), iii) or a rapid, reversible binding followed by a covalent modification of the enzyme (eg: aspirin). Selective COX-2 inhibitors exhibit time-dependent inhibition of COX-2 but not COX-1.

The two COX enzymes possess a long hydrophobic channel. At the entrance of the channel, Arg^{120}, Glu^{524}, Tyr^{355} and His^{90} form a network of hydrogen bonds that act as a gate to the binding site. NSAIDs generally bind between the upper portion of the COX channel located near Tyr^{385} and Arg^{120} which is present at the mouth of the COX channel as shown in Figure 1.0.6.

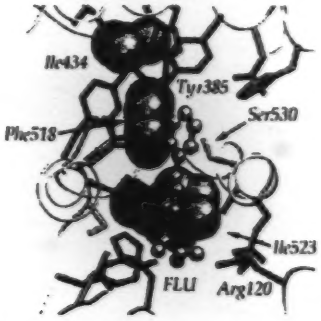
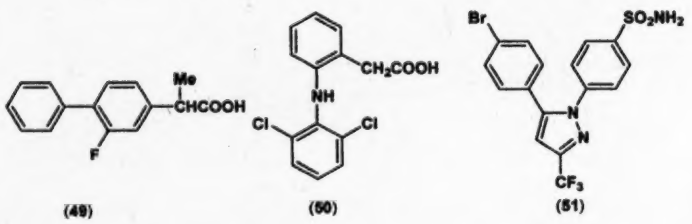


Figure 1.0.6: NSAID binding in the COX active site with flurbiprofen (yellow) bound in the ovine COX-1 active site channel (adapted from ref 89).



The carboxyl moiety of acidic NSAIDs such as flurbiprofen (**49**) and diclofenac (**50**) interacts with Arg^{120} in both COX isoforms, via hydrogen bonding or electrostatic interactions. The remaining ligand-protein interaction is hydrophobic. In the COX-2 active site, due to the presence of a smaller valine at amino acid residue position 523 (isoleucine in COX-1) and a valine (isoleucine in COX-1) subsitution at position 434 creates an extra pocket (secondary pocket) which is accessible in the COX-2 active site. This difference, increases the overall volume of COX-2 active site (394 $Å^3$) by almost 20% compared to COX-1 (316 $Å^3$).

Thus nonacidic selective COX-2 inhibitors can show enhanced binding to COX-2 due to reduced steric and ionic crowding at the mouth by Arg^{120}. The X-ray crystal structure of the diarylheterocyclic selective COX-2 inhibitor SC-558 (**51**) firmly established the structural basis for the COX-2 selectivity exhibited by this class of compounds (Figure 1.0.7).[21]

The *para*-SO_2NH_2 pharmacophore of the 1,5-diarylpyrazole **51** played a crucial role in COX-2 selectivity by insertion into the COX-2 secondary pocket to form favourable interactions with amino acid residues lining the secondary pocket such as His^{90},

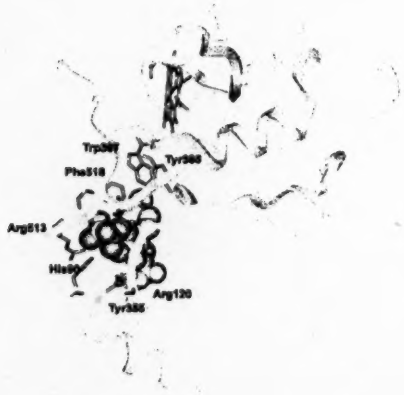


Figure 1.0.7: The ribbon diagram of the murine COX-2 enzyme with the diaryheterocyclic selective COX-2 inhibitor SC-558 (represented as space filling model) bound to the COX-2 active site.

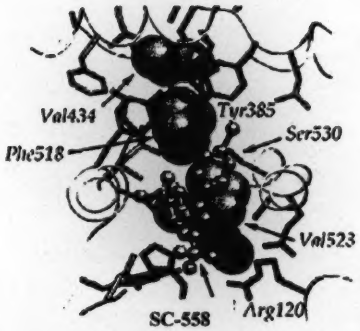


Figure 1.0.8: The diaryheterocyclic selective COX-2 inhibitor SC-558 (yellow colored) bound to the murine COX-2 active site. Amino acid residues Val^{434}, Val^{523} and Phe^{518} are shown as space filling (copper colored) models (adapted from ref 89).

Arg^{513}, Phe^{518} and Gln^{192} within the COX-2 active site. The C-5 *para*-bromophenyl ring of **51** is oriented towards the top of the COX-2 active site and undergoes hydrophobic contacts with Phe^{381}, Tyr^{385}, Phe^{513}, Trp^{387} and Leu^{384} (Figure 1.0.8).

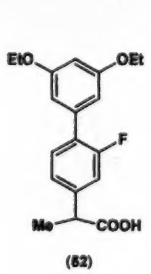
The CF_3 group at the 3-position of the central pyrazole ring binds to a hydrophobic pocket consisting of Met^{113}, Val^{116}, Val^{349}, Tyr^{355}, Leu^{359} and Leu^{531}. This X-ray crystal structure showed the importance of pharmacophores such as a SO_2NH_2, or a SO_2Me, substituent at the *para*-position of one of the phenyl rings in the design of diarylheterocyclic or diarylcarbocyclic selective COX-2 inhibitors.

1.4.1.1. Examples of successful application of molecular modeling (docking) experiments in the design of selective COX-2 inhibitors

A comparison of X-ray crystal structures for the two COX isoforms has identified structural differences within the two COX active sites. Computer modeling has helped to exploit these differences in the design of selective COX-2 inhibitors.

1.4.1.2. Design of COX-2 selectivity into the nonselective COX inhibitor flurbiprofen

Docking the nonselective COX inhibitor flurbiprofen (**49**) into the human COX-2 crystal structure showed a potential difference at the upper part of the COX active site. A conserved residue Leu^{384} was oriented differently in each isoform due to a sequence difference of the neighboring residue 503. In COX-1, residue 503 is phenylalanine whose larger size pushes the Leu^{384} side chain into the upper part of the active site. In contrast, due to the smaller Leu^{503}, in COX-2 the Leu^{384} is oriented away from the active site, thereby forming a small lipophilic alcove.



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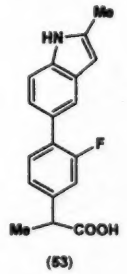
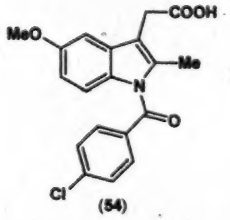


(53)

Scientists at Merck and Co exploited this difference in designing compound **52** possessing 3',5'-bisethoxy substituents that exhibited a 77 fold greater selectivity for COX-2 relative to COX-1 (COX-2 IC_{50} = 0.10 μM; COX-1 IC_{50} = 7.7 μM). Alternatively, compound **53** having a fused methylpyrrole ring system, occupied the lipophilic alcove present at the upper portion of the COX-2 active site. This resulted in a 45 fold greater selectivity for COX-2 than COX-1 (COX-2 IC_{50} = 0.23 μM; COX-1 IC_{50} = 10.5 μM).[90]

1.4.1.3. Design of COX-2 selectivity into the nonselective COX inhibitor indomethacin

Recently, COX-2 selectivity was incorporated into the nonselective COX inhibitor indomethacin (**54**) with the aid of computational experiments which resulted in the identification of indomethacin analogs with excellent in vitro COX-2 selectivity and in vivo activity profiles.[91]



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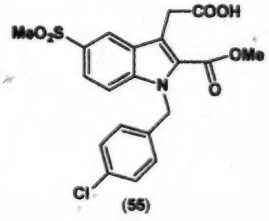


(55)

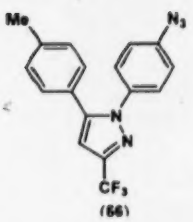
Accordingly, Palomer and coworkers, in an elegant study, described the design of indomethacin analogs as selective COX-2 inhibitors by incorporating a *para*-SO_2Me pharmacophore such that it interacted with the accessible COX-2 secondary pocket.

Molecular modeling studies confirmed that the *para*-SO_2Me pharmacophore of compound **55** was orientated within the COX-2 secondary pocket as shown in Figure 1.0.9. Compound **55** exhibited good in vitro COX-2 selectivity (COX-2 IC_{50} = 0.65 μM; COX-1 IC_{50} = 19 μM).[92]

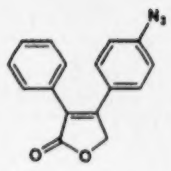
Figure 1.0.9: Overlay of the diaryheterocyclic selective COX-2 inhibitor SC-558 (yellow) with indomethacin analog (**55**) in green docked into the murine COX-2 active site. The sulfone moieties of SC-558 and **55** are in the same plane within the COX-2 secondary pocket (adapted from ref 92).

1.4.1.4. Replacement of sulfonamide and methylsulfone pharmacophores in celecoxib and rofecoxib by an azido bioisostere

The SO_2Me and SO_2NH_2 pharmacophores are known to induce COX-2 selectivity by insertion into the secondary pocket of COX-2 which is absent in COX-1. The secondary pocket present in COX-2 has been attributed to the presence of isoleucine (Ile^{523}) in COX-1 relative to the smaller valine (Val^{523}) in COX-2. Another critical difference between the two COX isozyme active sites is the presence of a histidine (His^{513}) in COX-1 relative to arginine (Arg^{513}) in the COX-2 active site.



(56)



(57)

Our research group has exploited this difference by replacing the respective *para*-SO_2NH_2 and *para*-SO_2Me pharmacophores present in celecoxib (Celebrex®) and rofecoxib (Vioxx®) with an azido substituent. The azido pharmacophore has the potential to undergo electrostatic (ion-ion) binding interactions with amino acid residues, particularly Arg^{513}, lining the secondary pocket of COX-2. The azide substituent is therefore a potentially new COX-2 pharmacophore to obtain COX-2 selectivity. A series of docking experiments of celecoxib and rofecoxib analogs showed that the *para*-azido pharmacophore was interacting with Arg^{513} and His^{90} within the COX-2 active site. Compounds **56** and **57** exhibited excellent in vitro COX-2 inhibitory potency and selectivity with good in vivo activity profiles.[93]

1.5.0.0. THERAPEUTIC APPLICATION OF SELECTIVE COX-2 INHIBITORS

1.5.1.0. Arthritis

Selective COX-2 inhibitors are currently used to relieve joint pain in patients with rheumatoid arthritis (RA) and osteoarthritis (OA). Patients with RA exhibit abundant COX expression, particularly the COX-2 isoform in synovial blood vessels, synovial lining cells and chondrocytes. Selective COX-2 inhibitors such as Celebrex® and Vioxx® have been shown in clinical trials to relieve pain and inflammation associated with RA as effectively as NSAIDs with significantly less adverse GI side effects.[94] OA is a debilitating, degenerative disease of the articular cartilage and synovial fluid which affects the elderly. The COX-2 enzyme was markedly induced in human tissues of osteoarthritis patients and is prominently expressed in the synovium, fibrocartilage of osteophytes, and in the blood vessels in the OA knee joint. Thus selective COX-2 inhibitors are now regularly used in treating the symptoms of OA.[95,96]

1.5.1.1. Cancer

Over the years several reports have shown that traditional NSAIDs such as sulindac and indomethacin exhibit protective effects against colorectal cancer.[97] The role of COX-2 in the development of colorectal tumors was first identified using COX-2 knock out mice which exhibited reduced numbers and size of polyps in animal tumor models.[98] The selective COX-2 inhibitor celecoxib received recent FDA approval as a pharmacologic adjunct in the management of familial adenomatous polyposis (FAP).[99]

Selective COX-2 inhibitors could also be potentially useful in the treatment of prostate cancer since the COX-2 enzyme is highly expressed in prostate cancer.[100] Lu and coworkers demonstrated that NS-398, a selective COX-2 inhibitor, induces apoptosis and down regulates bcl-2 expression in the human prostate cancer cell line LNCaP.[101] Recent studies have shown that the selective COX-2 inhibitor celecoxib induces apoptosis in human prostate cancer cell lines (PC-3)

expressing COX-2 by blocking Akt (antiapoptotic kinase) activation.[102]

Recent studies have shown that expression of cyclooxygenase-2 (COX-2) is elevated in gastric adenocarcinomas. These findings support the treatment of gastric carcinomas with selective COX-2 inhibitors as a chemotherapeutic modality.[103] In addition, COX-2 over expression has been described in human breast cancer where the COX-2 enzyme is present in about 40% of invasive breast carcinomas. These findings suggest that selective COX-2 inhibition may constitute an effective strategy for the treatment of breast cancers.[104]

1.5.1.2. Alzheimer's disease (AD)

The use of traditional NSAIDs has been associated with delaying the onset of AD in high risk families.[105] AD is associated with inflammatory conditions in the brain. Therefore, the protective effect provided by NSAIDs is consistent with their antiinflammatory activity. Up regulation of COX-2 expression was found in the frontal cortex in AD patients. Consequently, treatment of AD with selective COX-2 inhibitors may slow the progression of AD without causing GI side effects.[106] However, a recent study has shown that the selective COX-2 inhibitor Vioxx® failed to slow cognitive decline in patients with mild-to-moderate AD.[107]

1.5.1.3. Parkinson's disease (PD)

PD is a neurodegenerative disease wherein loss of dopaminergic transmission leads to rigidity, resting tremors and slowness of movement, ultimately leading to death. Since PD has an inflammatory component to its progression, studies on the role of COX-2 in PD neurodegeneration has shown that mice deficient with enzyme COX-2 were resistant to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced model of PD. These results showed that COX-2 plays an important role in MPTP-induced degeneration of dopaminergic neurons.[108]

Pzedborwski and coworkers recently described the pathological role of COX-2 in the development of PD. Their studies showed that COX-2 was up-regulated in brain dopaminergic neurons of PD patients and that the selective COX-2 inhibitor Vioxx® exhibited a neuroprotective effect in animal models of PD.[109]

1.6.0.0. REFERENCES

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2.0.0.0. OBJECTIVES OF THE RESEARCH

Nonsteroidal antiinflammatory drugs (NSAIDs) are invaluable agents for the treatment of RA and OA. Inhibition of the cyclooxygenase (COX) enzyme pathway is a hallmark feature of virtually all marketed NSAIDs. However, nonselective COX inhibition by NSAIDs such as indomethacin and ibuprofen can cause mechanism based side effects including dyspepsia and gastrointestinal ulceration/bleeding. The recently introduced selective COX-2 inhibitors Celebrex® and Vioxx® elicit efficient antiinflammatory and analgesic activities, and fewer adverse GI side effects. Selective COX-2 inhibitors are also proving to be very useful in the prophylactic treatment of a wide variety of cancers and neurodegenerative disorders.

The majority of selective COX-2 inhibitors belong to the tricyclic class. Extensive SAR studies for the tricyclic class of selective COX-2 inhibitors have shown that the nature and position of substituents on the central ring, and on the two vicinal aryl ring moieties attached to the central ring, are important determinants of the type of binding interactions within the COX-2 active site, which in turn dictates COX-2/COX-1 selectivity. Therefore, it was of interest to design a second generation tricyclic COX-2 inhibitors.

Accordingly, the primary objectives of this research encompass the design, synthesis and pharmacological evaluation of a diverse variety of tricyclic classes of compounds, to ultimately provide a more efficacious, mechanism-specific (selective), nonsteroidal COX-2 inhibitory antiarthritic agent that exhibits clinically acceptable antiinflammatory and analgesic activities.

Hypothesis 1: The spatial disposition of the 2,3-diphenyl rings relative to the C-C of the cyclopropane ring to which they are attached is important to COX-2 selectivity, where the function of the 3-membered ring containing the C-C is to provide the necessary geometry about the C-C, and that the COX-2 binding interaction is sensitive to both steric and electronic properties at all three positions of the cyclopropane ring.

Hypothesis 2: The spatial disposition of the 3,4-diphenyl rings relative to the C=C of the lactone ring to which they are attached is important to COX-2 selectivity, where the function of the 6-membered ring is to provide the necessary geometry about the C=C bond and that the primary function of the C-6 substituent is to orient the molecule in the primary binding site, such that the *p*-SO_2Me moiety inserts into the COX-2 secondary pocket.

Accordingly, a group of 1,1-dihalo-2,3-diphenylcyclopropanes, 6-alkyl, alkoxy or alkylthio substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones and 6-substituted-3,4,6-triphenylpyran-2-ones were prepared using a variety of synthetic methods that were evaluated in vitro to assess their COX-2 inhibitory and selectivity profiles. In vivo pharmacological evaluation was carried out using animal models to assess the antiinflammatory-analgesic potential (carrageenan-induced rat paw edema and 4% NaCl-induced abdominal constriction assays).

2.0.0.0. CHAPTER 1.0

Design, Syntheses and Evaluation of Novel 1,1-Dihalo-2,3-diphenylcyclopropanes as Potential Cyclooxygenase-2 (COX-2) Inhibitors with Analgesic-Antiinflammatory Activity

2.1.0.0. Introduction

Current interest in the design and development of selective cyclooxygenase-2 (COX-2) inhibitors is attributed to their reduced adverse effects on the gastrointestinal (GI) tract and renal system compared to traditional NSAIDS.[1-3] Celebrex® (celecoxib, **1**) was the first selective COX-2 inhibitor marketed for the treatment of both rheumatoid arthritis (RA) and osteoarthritis (OA).[4] More recently, Vioxx® (rofecoxib, **2**) has been introduced for the treatment of OA.[5]

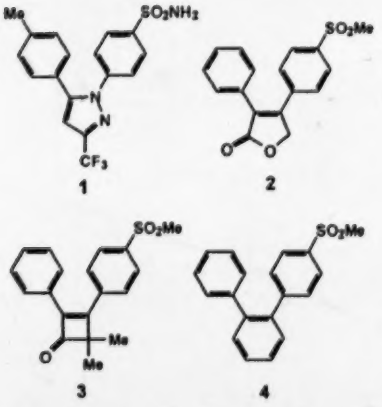
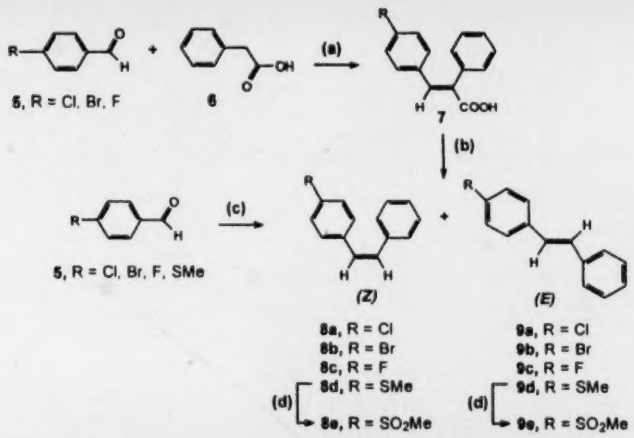


Chart 1.1: Structures of some selective cyclooxygenase-2 (COX-2) inhibitors having a central 4-, 5- or 6-membered ring system represented by Celecoxib (Celebrex®) (**1**), Rofecoxib (Vioxx®) (**2**), 3-(4-methanesulfonylphenyl)-2,2-dimethyl-4-phenylcyclobutenone (**3**), and 1-(4-methanesulfonylphenyl)-2-phenylbenzene (**4**).

A number of central ring templates have been successfully designed that, when appropriately functionalized, provide selective COX-2 inhibitory compounds. One common feature of these tricyclic molecules is 1,2-diaryl substitution to a central 4-, 5- or 6-membered ring system such as cyclobutene, pyrazole or 2-(5*H*)-furanone, or benzene, respectively (Chart 1.1). Structure-activity relationship (SAR) studies have shown that a -SO_2Me, -SO_2NH_2 or -F substituent at the *para*-position of a phenyl ring provides optimal COX-2 selectivity, inhibitory potency and oral activity.[6,7] Compounds with a smaller four-membered cyclobutenone ring system, in which the dihedral angle between the two aryl rings attached to the olefinic bond of the pharmacophore is larger relative to related five-membered rings, showed greater COX-2 selectivity.[8] In a previous study, we reported that fusion of a dichloro- or dibromocyclopropane moiety across the C_5-C_6 olefinic bond of a 1,4-dihydropyridine ring system afforded 4-benzoyl-5-phenyl-7-halo-2-azabicyclo[4.1.0]hept-3-enes that exhibited good antiinflammatory and analgesic activity.[9] Furthermore, 1,1-dichloro-2,3-diphenylcyclopropanes are also of interest since they have been reported to be potent antitubulin and antibreast cancer agents.[10] In the present study, we report the syntheses and biological evaluation of (*Z*)- and (*E*)-1,1-dihalo-2-(4-substituted-phenyl)-3-phenylcyclopropanes, in which a central 4-,5- or 6-membered ring has been



Scheme 1.1: Reagents and conditions: (**a**) Ac_2O, Et_3N, reflux 12 h; (**b**) quinoline, $2CuO\text{-}CrO_3$, reflux at 180-190°C, 2h; (**c**) $C_6H_5CH_2P^+(Ph)_3\ Cl^-$, 50% aqueous NaOH, 25°C, 30 min; (**d**) 50% aqueous Oxone® (potassium peroxymonosulfate) solution, MeOH, THF, 25°C, 2-3 h.

replaced by a 3-membered cyclopropane ring, designed as selective COX-2 inhibitors with analgesic-antiinflammatory activity. This design concept is based on a hypothesis that the spatial disposition [(*Z*) and (*E*)] of the 2,3-diaryl rings relative to the C-C of the cyclopropane ring to which they are attached could be an important determinant of COX-2 selectivity, and that the COX-2 binding interaction is sensitive to both electronic and steric properties of substituents at the *para*-position on the C-2 phenyl moiety. The vicinal diarylcyclopropane ring system is a good model to test this hypothesis.

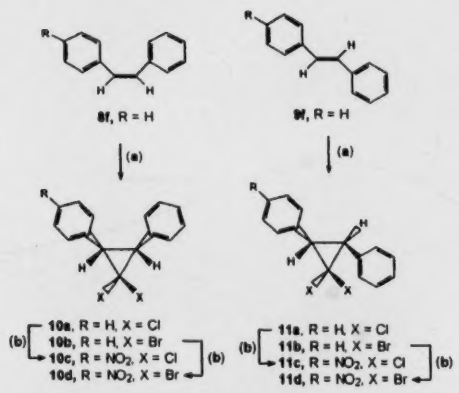
2.2.0.0. Chemistry

The *para*-substituted (*Z*)- and (*E*)-stilbenes (**8a-d** and **9a-d**), required for the synthesis of the target 1,1-dihalo-2,3-diphenylcyclopropanes, were prepared in good yield using the Perkin condensation[10] of a *para*-substituted-benzaldehyde (**5**, R = Cl, Br, F) with phenylacetic acid (**6**) in the presence of a base, and subsequent decarboxylation of the resulting cinnamic acid derivative (**7**) to yield a mixture of two separable ***Z*)-8a-d** and **(*E*)-9a-d** isomers in a ratio 5:1, respectively (see Scheme 1.1).

Alternatively, the Wittig reaction[14] of benzyltriphenylphosphonium chloride with a *para*-substituted-benzaldehyde (**5**, R = Cl, Br, F, SMe) afforded a mixture of the two separable **(*Z*)-8a-d** and **(*E*)-9a-d** stilbenes in a ratio of about 1.3:1, respectively. Oxidation of the **(*Z*)-8d** and **(*E*)-9d** methylthio stereoisomers using Oxone® afforded the respective **(*Z*)-8e**, or **(*E*)-9e**, methanesulfonyl derivative.

Nitration of the 1,1-dihalo-2,3-diphenylcyclopropanes [**(*Z*)-10a-b** or **(*E*)-11a-b**] by nitronium ion, generated in situ from copper (II) nitrate trihydrate in acetic anhydride,[22] afforded the respective 1,1-dihalo-2-(4-nitrophenyl)-3-phenylcyclopropane [***(Z)*-10-c-d**, or ***(E)*-11c-d**, Scheme 1.2].

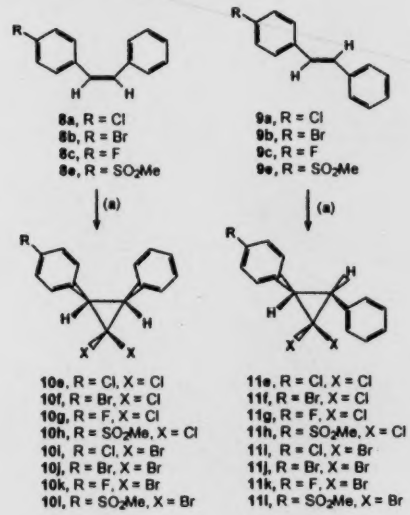
1,1-Dihalocyclopropanes derived from stilbenes can be prepared in good yields by the in situ generation of dihalocarbenes ($:CX_2$). Dihalocarbenes generated thermally from organomercurial reagents,[15] or a trihalomethane (CHX_3) in the presence of NaOH and a phase transfer catalyst,[16] undergo facile addition to olefinic bonds (Schemes 1.2 and 1.3).



Scheme 1.2: Reagents and conditions: **(a)** 50% aqueous NaOH, CHX_3 (X = Cl, Br), benzyltriethylammonium chloride, 25°C; **(b)** copper (II) nitrate trihydrate, Ac_2O, 25°C, 38-48 h.

Dihalocarbenes are less reactive species, relative to carbenes, that give a superior yield of cyclopropane product due to the absence of undesired insertion reactions.[23-25] Accordingly, the thermal in situ generation of dihalocarbenes ($:CX_2$, X = Cl, Br) from the Seyferth reagents $PhHgCBrCl_2$ or $PhHgCBr_3$ in the presence of a *para*-substituted (*Z*)- or (*E*)-stilbene **[(*Z*)-8a-c,e; (*E*)-9a-c,e]** afforded the respective *(Z)-*, or *(E)*-1,1-dichloro(or 1,1-dibromo)-2-(4-substitued-phenyl)-3-phenylcyclopropane **[(*Z*)-10e-l; (*E*)-11e-l]** in moderate to good yield (26-70%) as illustrated in Scheme 1.3.

A large number of ^1H NMR spectra for substituted-cyclopropanes have been reported. Our results are consistent with the fact that the magnitude of vicinal coupling constants for J_{cis} are always larger than J_{trans} for any given pair of cyclopropyl stereoisomers.[26-29] Accordingly, the (*Z*)-, or (*E*)-stereochemistry for this group of 1,1-dihalocyclopropanes was assigned based on the ^1H NMR chemical shift positions and coupling constants for the vicinal cyclopropyl C-2 and C-3 protons. In this respect, the resonances for the cyclopropyl vicinal hydrogens appeared as an AB quartet around δ 3 for the previously unreported *(Z)*-1,1-dihalo-2,3-diphenylcyclopropanes (**10d, 10h-l**) with coupling constants (*J* values) ranging from 11.6-12.2 Hz,[10] which clearly shows the nonequivalent nature of the vicinal cyclopropyl hydrogens.

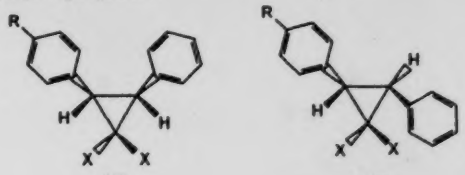

8a, R = Cl
8b, R = Br
8c, R = F
8e, R = SO2Me
9a, R = Cl
9b, R = Br
9c, R = F
9e, R = SO2Me
(a)
(a)
10e, R = Cl, X = Cl
10f, R = Br, X = Cl
10g, R = F, X = Cl
10h, R = SO2Me, X = Cl
10i, R = Cl, X = Br
10j, R = Br, X = Br
10k, R = F, X = Br
10l, R = SO2Me, X = Br
11e, R = Cl, X = Cl
11f, R = Br, X = Cl
11g, R = F, X = Cl
11h, R = SO2Me, X = Cl
11i, R = Cl, X = Br
11j, R = Br, X = Br
11k, R = F, X = Br
11l, R = SO2Me, X = Br

Scheme 1.3: Reagents and conditions: **(a)** $PhHgCBrCl_2$ (X = Cl), or $PhHgCBr_3$ (X= Br), benzene, 78-80°C, reflux, 8 h.

2.3.0.0. Results and discussion

A small group of *(Z)*- and *(E)*-1,1-dichloro(or dibromo)-2,3-diphenylcyclopropanes **[(*Z*)-10a-b; (*E*)-11a-b]** were prepared using a benzyltriethylammonium chloride catalyzed phase transfer dihalocyclopropanation ($:CX_2$) reaction of the **(*Z*)-8f** and **(*E*)-9f** stilbenes. The *para*-nitrophenyl derivatives **(*Z*)-10c-d** and **(*E*)-11c-d** were prepared by nitration of *(Z)*- and *(E)*-1,1-dichloro(or dibromo)-2,3-diphenylcyclopropanes **[(*Z*-10a-b, (*E*)-11a-b]** using copper nitrate and

Table 1.1: Antiinflammatory and Analgesic Activities, Molecular Volumes, and Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) Inhibition Activities for (*Z*)- and (*E*)-1,1-dihalo-2,3-diphenylcyclopropanes.



			IC_{50} (μM) [a]		Selectivity Index [b]	AI Activity [c]		Analgesic Activity [d]		Volume ($Å^3$)[e]
Cmpd	R	X	COX-1	COX-2		% Inhibition at 3 hours	% Inhibition at 5 hours	% Inhibition at 30 min.	% Inhibition at 60 min.	
(*Z*)-10a	H	Cl	–	–	–	23.4 ± 4.3	15.3 ± 3.2	57.0 ± 09.8	55.0 ± 6.1	227
(*E*)-11a	H	Cl	–	–	–	34.9 ± 12.3	09.3 ± 19.9	53.3 ± 15.7	49.6 ± 8.4	227
(*Z*)-10c	NO_2	Cl	> 200	214.43	–	30.3 ± 11.6	35.1 ± 9.7	56.7 ± 7.3	63.1 ± 7.6	247
(*E*)-11c	NO_2	Cl	278.80	80.52	3.5	–	–	–	–	247
(*Z*)-10e	Cl	Cl	–	–	–	15.1 ± 7.8	13.0 ± 8.3	65.6 ± 16.3	74.4 ± 5.9	240
(*E*)-11e	Cl	Cl	–	–	–	10.3 ± 7.6	9.8 ± 7.8	73.3 ± 7.0	69.2 ± 8.7	240
(*E*)-11f	Br	Cl	–	–	–	1.5 ± 7.6	Inactive	54.9 ± 6.4	63.4 ± 14.5	248
(*Z*)-10h	SO_2Me	Cl	> 100	> 200	–	23.4 ± 4.1	18.8 ± 6.0	44.2 ± 15.8	65.4 ± 8.1	273
(*E*)-11h	SO_2Me	Cl	0.59	3.04	–	30.9 ± 9.9	35.95 ± 5.2	71.6 ± 9.0	77.1 ± 4.5	273
(*Z*)-10l	SO_2Me	Br	–	–	–	03.9 ± 9.2	14.2 ± 9.4	54.6 ± 14.4	54.5 ± 10.6	279
(*E*)-11l	SO_2Me	Br	28.90	> 100	6.7	44.9 ± 8.1	37.2 ± 12.9	54.5 ± 13.2	73.1 ± 6.2	279
Aspirin			–	–	–	–	–	57.8 ± 2.8	–	154
Ibuprofen						43.8 ± 2.8	51.7 ± 3.6	–	–	212
Celecoxib			22.90	0.0567	> 404	79.9 ± 1.9	58.2 ± 1.8	31.7 ± 9.6	62.0 ± 7.3	298

[a] Values are means of two determinations and deviation from the mean is < 10% of the mean value. [b] In vitro COX-2 selectivity index (IC_{50} COX-1/IC_{50} COX-2). [c] Inhibitory activity in the rat 4% NaCl -induced abdominal constriction assay. The results are expressed as mean ± SEM (n = 4–6) following a 50 mg/kg intraperitoneal dose of the test compound. [d] Inhibitory activity on carrageenan-induced rat paw edema. The results are expressed as mean ± SEM (n = 4–6) following a 50 mg/kg oral dose of the test compound. [e] The volume of the molecule after minimization using the PM3 forcefield, was calculated using the Alchemy 2000 program.

acetic anhydride (Scheme 1.3). A related group of *(Z)*- and *(E)*-1,1-dihalo-2-(4-substituted-phenyl)-3-phenylcyclopropanes **[(*Z*)-10e-l, (*E*)-11e-l]** that possess either dichloro or dibromo substituents at C-1 of the cyclopropane ring were synthesized by the stereospecific addition of dihalocarbenes ($:CX_2$ generated from $PhHgCBrCl_2$ or $PhHgCBr_3$) to the olefinic bond of *para*-phenyl-substituted (F, Cl, Br, SO_2Me) (*Z*)- and (*E*)- stilbenes **[(*Z*)-8a-c,e; (*E*)-9a-c, e]** as illustrated in Scheme 1.3.

In vivo analgesic activity, determined using the 4% NaCl-induced writhing (abdominal constriction) assay, showed that this group of 1,1-dihalo-2-(4-substituted-phenyl)-3-phenylcyclopropanes **[(*Z*)-10, (*E*)-11)** inhibited writhing by 44-77% relative to the reference drugs aspirin and celecoxib (58 and 62% inhibition at 30 and 60 minutes post-drug administration, respectively) as summarized in Table 1.1.

In vivo antiinflammatory activity, determined using the carrageenan-induced rat paw edema assay, showed that these 1,1-dihalo-2-(4-substituted-phenyl)-3-phenylcyclopropane derivatives **[(*Z*)-10, (*E*)-11]** inhibited inflammation by 1.5-45% at 3 hours, and 9-37% at 5 hours, post-drug administration relative to reference drug ibuprofen (44 and 52% inhibition at 3 and 5 hours, respectively (Table 1.1).

In vitro COX-1 and COX-2 inhibition studies showed that *(E)*-1,1-dichloro-2-(4-nitrophenyl)-3-phenylcyclopropane (**11c**) inhibited COX-1 (IC_{50} = 278.8 μM) and COX-2 (IC_{50} = 80.5 μM) to provide a COX-2 selectivity index of 3.5. In contrast, *(E)*-1,1-dichloro-2-(4-methanesulfonylphenyl)-3-phenylcyclopropane (**11h**) was a more potent inhibitor of both COX-1 (IC_{50} = 0.59 μM) and COX-2 (IC_{50} = 3.04 μM), but it was more selective for the COX-1 isozyme (Table 1.1).

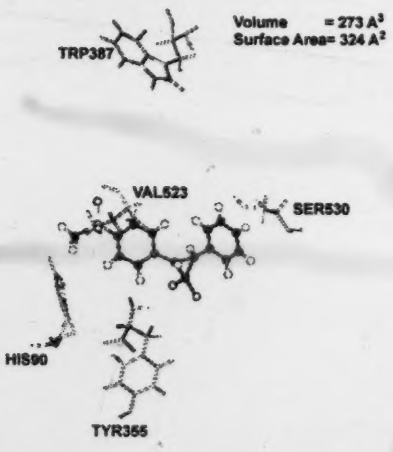


Figure 1.1: Docking of *(E)*-1,1-dichloro-2-(4-methanesulfonylphenyl)-3-phenylcyclopropane (**11h**) (ball and stick) on the active site of murine COX-2 (line and stick) ($E_{intermolecular}$ = -39.08 Kcal/mol.

Docking *(E)*-1,1-dichloro-2-(4-methane sulfonylphenyl)-3-phenylcyclopropane (**11h**) on the active site of murine COX-2 (Figure 1.1) indicates that the 1,1-dichloro substituents are oriented in the direction of the mouth of the channel towards Arg^{120}, and that the C-2 $MeSO_2$ moiety is oriented towards the apex of the active site with the S-atom of the $MeSO_2$ substituent positioned about 6.56 Å inside the entrance to the secondary pocket (Val^{523}). In contrast, the S-atom of the $MeSO_2$ substituent of the (*Z*)-stereoisomer (**10h**) is much further removed from the entrance to the secondary pocket of COX-2 (Figure 1.2).

Cyclopropyl C-C bonds mimic a C=C bond due to their ability to conjugate with an adjacent olefinic bond,[30] and the hybridization of a cyclopropyl C-C bond is intermediate in character between sigma (σ) and pi (π) bonds. In the 1,1-dihalo-2-(4-substituted-phenyl)-3-phenylcyclopropane class of compounds, the two phenyl substituents are bent out of the plane of the planar cyclopropane ring since they are attached to a sp^3 hybridized carbon that results in either a (*Z*)- or (*E*)-stereochemical orientation. Furthermore, the *geminal* C-1 dihalo substituents in (*Z*)- and (*E*)-1,1-dihalo-2,3-diphenyl cyclopropanes are oriented such that one halogen atom is above, and the other halogen atom is below, the plane of the planar cyclopropane ring. It was therefore of interest to investigate the effect which various substituents (H, Cl, Br, F, NO_2, SO_2Me) at the *para*-position of the C-2 phenyl ring present in (*Z*)- and (*E*)-1,1-dihalo-2,3-diphenylcyclo propane stereoisomers has upon analgesic-antiinflammatory activity, and COX-2 selectivity. Acquisition of this type of biological data should indicate

whether a central cyclopropane ring scaffold is a suitable template for the design of selective COX-2 inhibitors.

Analgesic structure-activity data, determined using the 4% NaCl-induced abdominal constriction assay showed that this group of **(*Z*)-10** and **(*E*)-11** 1,1-dihalo-2-(4-substituted-phenyl)-3-phenylcyclopropanes inhibited writhing by 44-73% at 30 minutes, and 49-77% at 60 minutes, post-drug administration, relative to the reference drugs aspirin and celecoxib (58 and 32% inhibition at 30 minutes post-drug administration) for a 50 mg/kg intraperitoneal dose (Table 1.1). The relative analgesic potency profile with respect to substituent variation at the *para*- position of the C-2 phenyl ring was: **(*Z*)-10** series of compounds at 30 minutes, Cl > NO_2 ≈ H > SO_2Me; **(*E*)-11** series at 30 minutes, Cl ≈ SO_2Me > Br ≈ H; **(*Z*)-10** series at 60 minutes, H ≈ Cl ≥ SO_2Me ≈ NO_2; **(*E*)-11** series at 60 minutes, SO_2Me > Cl ≥ Br > H. Overall, the Cl and $MeSO_2$ *para*-phenyl substituents generally provided superior analgesic activity. The most active compound, *(E)*-1,1-dichloro-2-(4-methanesulfonylphenyl)-3-phenylcyclopropane **(11h)** exhibited good analgesic activity at 30 minutes (72% inhibition) and 60 minutes (77% inhibition) post-drug administration, respectively.

Structure-activity relationships, acquired using the antiinflammatory rat paw edema assay, showed that this group of **(*Z*)-10** and **(*E*)-11** 1,1-dihalo-2-(4-substituted-phenyl)-3-phenylcyclopropane compounds exhibit antiinflammatory activity in the inactive-to-moderate activity range (1.5-45% inhibition) with respect to the *para*-phenyl substituent at a 50 mg/kg oral dose (see Table 1.1). The antiinflammatory potency order for the **(*Z*)-10** series of compounds was NO_2 > $MeSO_2$ ≈ H ≥ Cl, and for the **(*E*)-11** series of compounds was H ≥ $MeSO_2$ > Cl ≈ Br. (*E*)-1,1-dibromo-2-(4-methanesulfonylphenyl)-3-phenylcyclopropane **(11l)** was the most active antiinflammatory agent (45% and 37% reduction in inflammation at 3 and 5 hours post-drug administration, respectively). Within the *para*-methanesulfonylphenyl group of compounds, the (*E*)-stereoisomers **(11h, 11l)** were more active than the corresponding (*Z*)-stereoisomers **(10h, 10l)**.

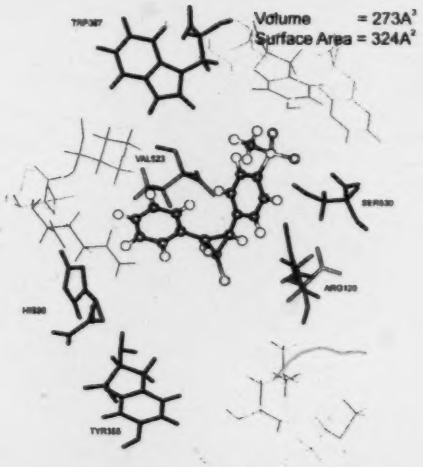


Figure 1.2: Docking of (*Z*)-1,1-dichloro-2-(4-methanesulfonylphenyl-3-phenylcyclopropane **(10h)** (ball and stick) on the active site of murine COX-2 (line and stick) ($E_{intermolecular}$ = -8.41 Kcal/mol).

The major difference between the binding sites for COX-1 and COX-2 is at position 523 where COX-2 has the amino acid residue Val in place of Ile for COX-1. This difference produces a secondary pocket extending off the primary binding site in COX-2 that is absent in COX-1.

Consequently, the combined volume (394 $Å^3$) of the primary binding site and the secondary pocket in COX-2 is about 25% larger than the volume of the COX-1 (316 $Å^3$) binding site.[31] This difference in volume, and the presence of the secondary pocket in COX-2, can be exploited by varying the volume of the drug and the placement of substituents possessing different electronic and steric properties (Cl, Br, F, NO_2, SO_2Me) at the *para* position of one of the aryl rings present in (*Z*)- and (*E*)-1,1-dihalo-2,3-diphenylcyclopropanes.[9,32] Compounds **10-11** have volumes in the range of 227-279 $Å^3$, relative to the selective COX-2 inhibitor celecoxib (298 $Å^3$), and the nonselective COX-1/COX-2 inhibitor ibuprofen (212 $Å^3$) as listed in Table 1.1.

A comparison of the ability of the **(*Z*)-10c** and **(*E*)-10c** (R = NO_2), and the **(*Z*)-10h** and **(*E*)-11h** (R = $MeSO_2$) stereoisomers to inhibit COX-1 and COX-2 indicates that the (*Z*)-stereoisomers (**10c**, **10h**) are inactive inhibitors of both COX-1 and COX-2 (IC_{50} > 100 μM). Although the (*E*)-stereoisomer (**11c**, R = NO_2) was a moderately selective COX-2 inhibitor [COX-2 selectivity index (SI) = 3.5], **(*E*)-11c** remains a weak inhibitor of COX-2 (IC_{50} = 80.5 μM). In contrast, **(*E*)-11h** (R = $MeSO_2$) was a much more potent inhibitor of both COX-1 (IC_{50} = 0.59 μM) and COX-2 (IC_{50} = 3.04 μM), but it is more selective for COX-1 than COX-2. These results suggest that (*E*)-1,1-dihalo-2-(4-substituted-phenyl)-3-phenylcyclopropanes (**11**) bind more favourably to the active site of either COX-1 or COX-2 than the corresponding **(*Z*)-10** isomer.

It was therefore of interest to dock the **(*E*)-11h**, and **(*Z*)-10h**, 1,1-dichloro-2-(4-methanesulfonylphenyl)-3-phenylcyclopropanes stereoisomers on the active site of the human COX-2 isozyme to determine their orientation in the active site which may help to explain their difference in COX-2 binding efficacy. Docking the **(*E*)-11h** stereoisomer in the active site of COX-2 (Figure 1.1) showed that it binds in the center of the active site with the 1,1-dichloro substituents oriented in the direction of the mouth of the channel (Arg^{120}). The $MeSO_2$ moiety is oriented towards the apex of the active site, and the *S*-atom of the $MeSO_2$ group is positioned about 6.56 Å inside the entrance to the secondary pocket of COX-2 (Val^{523}). A similar docking study for the **(*Z*)-10h** stereoisomer (Figure 1.2) shows that the unsubstituted C-3 phenyl ring moiety is oriented toward the COX-2 secondary pocket, and that the *S*-atom of the $MeSO_2$ moiety is now much closer to the Ser^{530} *O*H group (4.02 Å). These molecular modeling (docking) studies suggest that the $MeSO_2$ moiety present in **(*E*)-11h** is more suitably positioned, relative to the $MeSO_2$ moiety in **(*Z*)-10h**, since it is inserted into the secondary pocket of COX-2 near Val^{523}. One potential drug design approach to increase the COX-2 selectivity and/or inhibitory potency of the **(*E*)-11h** stereoisomer could involve selective replacement of one of the 1,1-dichloro substituents by a larger substituent such as CF_3 or an alkyl substituent (Et, cyclopropyl, *i*-Bu, *t*-Bu) which would increase the overall size of the molecule to take advantage of the larger volume of the COX-2 binding site and its associated secondary pocket. More particularly, substituents of this latter type offer a method to reorient the $MeSO_2$ moiety present in **(*Z*)-10h** such that it is properly positioned to insert deep into the secondary binding pocket present in

COX-2 that may result in selective COX-2 binding, and potent COX-2 inhibition.

2.4.0.0. Conclusions

The results of this investigation shows that (i) a 3-membered cyclopropane ring can serve as a potential ring template in the design of tricyclic COX-2 inhibitors that exhibit in vivo antiinflammatory-analgesic activity (ii) that the COX-2 binding interaction is governed by steric and electronic properties at the *para*-position on the C-2 phenyl ring, and (iii) that the molecular modeling studies help in understanding the binding interactions of 1,1-dihalo-2,3-diarylcyclopropanes in the COX-2 active site.

2.5.0.0. Experimental section

General. Melting points were determined using a Buchi capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Nicolet 550 Series II Magna FT-IR spectrometer. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR, ^{19}F NMR) spectra were recorded on a Bruker AM-300 spectrometer. Elemental analyses were performed for C, H and N (MicroAnalytical Service Laboratory, Department of Chemistry, University of Alberta). The volume (Å^3) of compounds was calculated using an IBM computer with the Alchemy 2000 Program (Version 2.0) from SciVision Inc., after minimization using the MM3 force field. Molecular modeling experiments were performed on an Indigo 2 R4400 SGI workstation using the Insight II software modules Builder, Discover, Biopolymer and Docking (Molecular Simulations Inc., San Diego, CA). Preparative thin layer chromatography (PTLC) was performed using Camag Kieselgel DF-5 plates, 1.0 mm in thickness. Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70-230 mesh). Compounds **8a-d** and **9a-d** were synthesized (see procedures illustrated in Scheme 1.2) according to the reported procedures.[10-14] Phenyl(bromodichloro methyl)mercury ($PhHgCBrCl_2$) and phenyl(tribromomethyl) mercury were prepared according to the literature method.[15] Compounds **10a-c** and **11a-b** were synthesized using the reported methods as illustrated in Scheme 1.3.[16-18] All other reagents were purchased from Aldrich Chemical (Milwaukee, WI). In vitro COX-1 and COX-2 inhibition assays were performed using enzyme immunoassay (EIA) kits purchased from Cayman Chemical, Ann Arbor, MI. Male Sprague-Dawley rats, used in the antiinflammatory and analgesic screens, were supplied by Animal Health Services, University of Alberta. All experiments involving animals were carried out using protocols approved by the Animal Welfare Committee, University of Alberta.

General Procedure for the Synthesis of *(Z)*-4-Methanesulfonylstilbene (8e) and *(E)*-4-Methanesulfonylstilbene (9e). An aqueous solution of Oxone® (50% w/v, 6.0 mmol) was added drop wise with stirring to a solution of either ***(Z)*-8d**, or ***(E)*-9d**, (0.47 g, 2.0 mmol) in THF (15 mL) and MeOH (15 mL) at 0 °C, and the reaction was allowed to proceed at 25 °C for 2-3 h. The reaction mixture was concentrated in vacuo, diluted with water (25 mL), and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extracts were washed with brine (20 mL) and the organic fraction was dried (Na_2SO_4). Removal of the solvent in vacuo afforded a white solid that was purified by silica gel column chromatography using CH_2Cl_2-petroleum ether (9:1, v/v) as eluent to afford ***(Z)*-8e**,

or **(*E*)-9e**, that crystallized upon standing in a refrigerator.

Product (*Z*)-8e: Yield, 70%; mp 140-142 °C; IR (KBr): 1305, 1156 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.05 (s, 3H, SO_2CH_3), 6.58 (d, J = 12.2 Hz, 1H, Ph-C*H*=CH-), 6.77 (d, J = 12.2 Hz, 1H, Ph-CH=C*H*-), 7.11-7.27 (m, 5H, phenyl hydrogens), 7.40 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.77 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{15}H_{14}O_2S$): C, H.

Product (*E*)-9e: Yield, 82%; mp 179-181 °C; IR (KBr): 1305, 1153 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.08 (s, 3H, SO_2CH_3), 7.11 and 7.16 (two s, 1H each, -C*H*=C*H*-), 7.27-7.57 (m, 5H, phenyl hydrogens), 7.67 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.91 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). The ^1H NMR spectral data was identical to that reported for **(*E*)-9e** obtained by oxidation of a mixture of **(*Z*)-8d** and **(*E*)-9d** using *meta*-chloroperbenzoic acid and then recrystallization of **(*E*)-9e** from the product mixture.[8,12]

General Procedure for the Synthesis of (*Z*)-1,1-Dibromo-2-(4-nitrophenyl)-3-phenylcyclopropane (10d), (*E*)-1,1-Dichloro-2-(4-nitrophenyl)-3-phenylcyclopropane (11c), and (*E*)-1,1-Dibromo-2-(4-nitrophenyl)-3-phenylcyclopropane (11d). A mixture of copper (II) nitrate trihydrate (0.56 g, 2.3 mmol) in Ac_2O (4.2 mL) was stirred at 25 °C for 1.5 h, the respective 1,1-dihalo-2,3-diphenylcyclopropane (**10b**, **11a** or **11b**, 1.1 mmol) was added, and the reaction was allowed to proceed at 25 °C for 14 h. An additional aliquot of copper (II) nitrate (0.28 g, 1.15 mmol) was added, and the reaction was allowed to proceed for a further 24 h at 25 °C with stirring. Water (20 mL) was added, the mixture was extracted with EtOAc (2 x 20 ml), the combined organic extracts were washed consecutively with 10% $NaHCO_3$ solution (2 x 20 mL), water (20 mL), and the organic fraction was dried (Na_2SO_4). Removal of the solvent in vacuo gave a brown oil that was purified by silica gel column chromatography using EtOAc-hexane (1:3, v/v) as eluent to afford the respective product (**10d**, **11c** or **11d**) as a pale yellow oil that solidified upon storage after several days at 0-5 °C.

Product 10d: Yield, 45%; mp 133-135 °C; IR (KBr): 1523, 1349 (NO_2), 3067, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.36 (d, J = 11.6 Hz, 1H, cyclopropyl, H-3), 3.47 (d, J = 11.6 Hz, 1H, cyclopropyl, H-2), 6.98-7.09 (m, 2H, phenyl H-2, H-6), 7.20 (d, J = 8.4 Hz, 2H, 4-nitrophenyl H-2, H-6), 7.25-7.27 (m, 3H, phenyl H-3, H-4, H-5), 8.14 (d, 2H, J = 8.4 Hz, 4-nitrophenyl H-3, H-5). Anal. ($C_{15}H_{11}Br_2NO_2$): C, H.

Product 11c: Yield, 31%; mp 88-91 °C; IR (KBr): 1518, 1346 (NO_2), 3050, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.22 (d, J = 9.1 Hz, 1H, cyclopropyl H-3), 3.25 (d, J = 9.1 Hz, 1H, cyclopropyl H-2), 7.26-7.29 (m, 2H, phenyl H-2, H-6), 7.36-7.40 (m, 3H, phenyl H-3, H-4, H-5), 7.54 (d, J = 8.5 Hz, 2H, 4-nitrophenyl H-2, H-6), 8.25 (d, J = 8.5 Hz, 2H, 4-nitrophenyl H-3, H-5). Anal. ($C_{15}H_{11}Cl_2NO_2$): C, H.

Product 11d: Yield, 42%; mp 120-123 °C, IR (KBr): 1518, 1353 (NO_2), 3050, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.27 (d, J = 9.1 Hz, 1H, cyclopropyl H-3), 3.30 (d, J = 9.1 Hz,

1H, cyclopropyl H-2), 7.26-7.27 (m, 2H, phenyl H-2, H-6), 7.40-7.42 (m, 3H, phenyl H-3, H-4, H-5), 7.55 (d, J = 8.4 Hz, 2H, 4-nitrophenyl H-2, H-6), 8.26 (d, J = 8.4 Hz, 2H, 4-nitrophenyl H-3, H-5). Anal. ($C_{15}H_{11}Br_2NO_2$): C, H.

General Procedure for the Synthesis of (*Z*)-1,1-Dihalo-2-(4-substituted-phenyl)-3-phenylcyclopropanes (10e-l) and (*E*)-1,1-Diahalo-2-(4-substituted-phenyl)-3-phenylcyclopropanes (11e-l). $PhHgCBrCl_2$ or $PhHgCBr_3$ (2.2 mmol) was added to a solution of the selected *para*-substituted (Cl, Br, F or SO_2Me) **(*Z*-)-8a-c,e**, or **(*E*)-9a-c,e**, stilbene derivative (2.2 mmol) in dry benzene (20 mL) under a nitrogen atmosphere with stirring, and the reaction mixture was refluxed for a total reaction time of 8 h at 78-80 °C during which time an additional aliquot of $PhHgCBrCl_2$ or $PhHgCBr_3$ (2.2 mmol) was added every 2 h. After cooling the reaction mixture to 25 °C, the precipitated PhHgBr was removed by filtration, and the solvent was removed in vacuo to afford a brown oil which was purified by preparative thin layer chromatography (PTLC) using CH_2Cl_2-petroleum ether (1:9, v/v), or EtOAc-hexane (1:3, v/v) as the development solvent. The physical and spectral data for the previously unreported **(*Z*)-10h-l** and **(*E*)-11e-l** products prepared using this general method are listed below.

***(Z)*-1,1-Dichloro-2-(4-methanesulfonylphenyl)-3-phenylcyclopropane (10h).** Product **10h** was prepared by reaction of **8e** with $PhHgCBrCl_2$. Yield, 50%; mp 142-144 °C; IR (KBr): 1303, 1152 (SO_2), 3046, 3020 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.09 (s, 3H, SO_2CH_3), 3.33 (d, J = 11.6 Hz, 1H, cyclopropyl H-3), 3.43 (d, J = 11.6 Hz, 1H, cyclopropyl H-2), 7.03-7.06 (m, 2H, phenyl H-2, H-6), 7.17 (d, J = 8.2 Hz, 2H, 4-methanesufonylphenyl H-2, H-6), 7.27-7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.78 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 39.1 (cyclopropane C-3), 39.7 (cyclopropane C-2), 44.4 (SO_2CH_3), 64.5 (cyclopropane C-1), 126.6, 127.7, 128.2, 130.6 (phenyl *C*H carbons, 4-methanesulfonylphenyl C-2 and C-6), 130.7 (4-methanesulfonylphenyl C-4), 131.6 (4-methanesulfonylphenyl C-3, C-5), 138.6 and 139.3 (4-methanesulfonylphenyl C-1 and phenyl C-1). Anal. ($C_{16}H_{14}Cl_2O_2S.1/3H_2O$): C, H.

***(Z)*-1,1-Dibromo-2-(4-chlorophenyl)-3-phenylcyclopropane (10i).** Product **10i** was synthesized by reaction of **8a** with $PhHgCBr_3$. Yield: 30% (oil); IR (film): 3058, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.34 (d, J = 11.9 Hz, 1H, cyclopropyl H-3), 3.41 (d, J = 11.9 Hz, 1H, cyclopropyl H-2), 6.96 (d, J = 8.2 Hz, 2H, 4-chlorophenyl H-2, H-6), 7.05-7.07 (m, 2H, phenyl H-2, H-6), 7.21 (d, J = 8.2 Hz, 2H, 4-chlorophenyl H-3, H-5), 7.27-7.29 (m, 3H, phenyl H-3, H-4, H-5). Anal. ($C_{15}H_{11}Br_2Cl.1/3H_2O$): C, H.

***(Z)*-1,1-Dibromo-2-(4-bromophenyl)-3-phenylcyclopropane (10j).** Product **10j** was prepared by reaction of **8b** with $PhHgCBr_3$. Yield, 34% (oil); IR (film): 3052, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.31 (d, J = 12.2 Hz, 1H, cyclopropyl H-3), 3.40 (d, J = 12.2 Hz, 1H, cyclopropyl H-2), 6.89 (d, 2H, J = 8.2 Hz, 4-bromophenyl H-2, H-6), 7.05-7.07 (m, 2H, phenyl H-2, H-6), 7.20-7.28 (m, 3H, phenyl H-3, H-4, H-5), 7.36 (d, J = 8.2 Hz, 2H, 4-bromophenyl

H-3, H-5). Anal. ($C_{15}H_{11}Br_3.1/2H_2O$): C, H.

***(Z)*-1,1-Dibromo-2-(4-fluorophenyl)-3-phenylcyclopropane (10k).** Product **10k** was obtained from the reaction of **8c** with $PhHgCBr_3$. Yield: 32% (viscous oil); IR (film): 3050, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.26 (d, J = 11.9 Hz, 1H, cyclopropyl H-3), 3.32 (d, J = 11.9 Hz, 1H, cyclopropyl H-2), 6.87-7.12 (m, 4H, 4-fluorophenyl hydrogens), 7.23-7.38 (m, 5H, phenyl hydrogens); ^{19}F NMR ($CDCl_3$): δ 47.45 (dddd, J_{FCCH} = 8.5 Hz, J_{FCCCH} = 4.9 Hz, 1F). Anal. ($C_{15}H_{11}Br_2F.1/2H_2O$): C, H.

***(Z)*-1,1-Dibromo-2-(4-methanesulfonylphenyl)-3-phenylcyclopropane (10l).** Product **10l** was synthesized by the reaction of **8e** with $PhHgCBr_3$. Yield, 70% (viscous oil); mp 122-125 °C; IR (film): 1306, 1152 (SO_2), 3050, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.10 (s, 3H, SO_2CH_3), 3.42 (d, J = 11.9 Hz, 1H, cyclopropyl H-3), 3.52 (d, J = 11.9 Hz, 1H, cyclopropyl H-2), 7.20-7.29 (m, 2H, phenyl H-2, H-6), 7.35- 7.47 (m, 3H, phenyl H-3, H-4, H-5), 7.59 (d, J = 8.2 Hz, 2H, 4-methanesufonylphenyl H-2, H-6), 7.98 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 39.1 (cyclopropane C-3), 39.7 (cyclopropane C-2), 44.4 (SO_2CH_3), 64.5 (cyclopropane C-1), 126.6, 127.7, 128.1, 130.0 (phenyl *C*H carbons, 4-methanesulfonylphenyl C-2 and C-6), 130.7 (4-methanesulfonylphenyl C-4), 131.6 (4-methanesulfonylphenyl C-3, C-5), 133.5 (phenyl C-1), 138.6 (4-methanesulfonylphenyl C-1). Anal. ($C_{16}H_{14}Br_2O_2S.1/3H_2O$): C, H.

***(E)*-1,1-Dichloro-2-(4-chlorophenyl)-3-phenylcyclopropane (11e).** Product **11e** was prepared by reaction of **9a** with $PhHgCBrCl_2$. Yield, 42%; mp 73-75 °C; IR (KBr): 3050, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.19 (d, J = 11.3 Hz, 1H, cyclopropyl H-3), 3.23 (d, J = 11.3 Hz, 1H, cyclopropyl H-2), 7.25-7.28 (m, 2H, phenyl H-2, H-6), 7.30-7.32 (m, 3H, phenyl H-3, H-4, H-5), 7.33-7.36 (m, 2H, 4-chlorophenyl H-2, H-6), 7.37-7.44 (m, 2H, 4-chlorophenyl H-3, H-5). Anal. ($C_{15}H_{11}Cl_3.1/2H_2O$): C, H.

***(E)*-1,1-Dichloro-2-(4-bromophenyl)-3-phenylcyclopropane (11f).** Product **11f** was synthesized by reaction of **9b** with $PhHgCBrCl_2$. Yield, 41% (viscous oil); IR (film): 3050, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.20 (d, J = 11.6 Hz, 1H, cyclopropyl H-3), 3.30 (d, J = 11.6 Hz, 1H, cyclopropyl H-2), 6.86 (d, J = 8.2 Hz, 2H, 4-bromophenyl H-2, H-6), 6.97-7.03 (m, 2H, phenyl H-2, H-6), 7.19-7.22 (m, 3H, phenyl H-3, H-4, H-5), 7.34 (d, J = 8.2 Hz, 2H, 4-bromophenyl H-3, H-5). Anal. ($C_{15}H_{11}BrCl_2.1/2H_2O$): C, H.

***(E)*-1,1-Dichloro-2-(4-fluorophenyl)-3-phenylcyclopropane (11g).** Product **11g** was prepared by reaction of **9c** with $PhHgCBrCl_2$. Yield: 27% (viscous oil); IR (film): 3046, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.12 (d, J = 11.6 Hz, 1H, cyclopropyl H-3), 3.18 (d, J = 11.6 Hz, 1H, cyclopropyl H-2), 6.90-7.05 (m, 4H, 4-fluorophenyl hydrogens), 7.19-7.30 (m, 5H, phenyl hydrogens); ^{19}F NMR ($CDCl_3$): δ 47.01 (dddd, J_{FCCH} = 9.2 Hz, J_{FCCCH} = 6.1 Hz, 1F). Anal. ($C_{15}H_{11}Cl_2F.1/3H_2O$): C, H.

***(E)*-1,1-Dichloro-2-(4-methanesulfonylphenyl)-3-**

phenylcyclopropane (11h). Product **11h** was synthesized by reaction of **9e** with $PhHgCBrCl_2$. Yield, 35%; mp 127-129 °C; IR (KBr): 1303, 1152 (SO_2), 3050, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.09 (s, 3H, SO_2CH_3), 3.26 (d, J = 9.4 Hz, 1H, cyclopropyl H-3), 3.30 (d, J = 9.4 Hz, 1H, cyclopropyl H-2), 7.27-7.28 (m, 2H, phenyl H-2, H-6), 7.33-7.45 (m, 3H, phenyl H-3, H-4, H-5), 7.58 (d, J = 8.5 Hz, 2H, 4-methanesufonylphenyl H-2, H-6), 7.97 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 39.7 (cyclopropane C-3), 40.3 (cyclopropane C-2), 44.5 (SO_2CH_3), 64.66 (cyclopropane C-1), 127.7, 128.0, 128.5, 128.7 (phenyl *C*H carbons, 4-methanesulfonylphenyl C-2, C-6), 129.9 (4-methanesulfonylphenyl C-3, C-5), 133.5 (phenyl C-1), 139.9 and 140.7 (4-methanesulfonylphenyl C-1 and C-4). Anal. ($C_{16}H_{14}Cl_2O_2S$): C, H.

***(E)*-1,1-Dibromo-2-(4-chlorophenyl)-3-phenylcyclopropane (11i).** Product **11i** was obtained from the reaction of **9a** with $PhHgCBr_3$. Yield, 28%; mp 95-97 °C; IR (KBr): 3050, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.22 (d, J = 9.2 Hz, 1H, cyclopropyl H-3), 3.26 (d, J = 9.2 Hz, 1H, cyclopropyl H-2), 7.26-7.29 (m, 2H, phenyl H-2, H-6), 7.31-7.33 (m, 3H, phenyl H-3, H-4, H-5), 7.36 (d, J = 8.8 Hz, 2H, 4-chlorophenyl H-2, H-6), 7.37-7.42 (m, 2H, 4-chlorophenyl H-3, H-5). Anal. ($C_{15}H_{11}Br_2Cl$): C, H.

***(E)*-1,1-Dibromo-2-(4-bromophenyl)-3-phenylcyclopropane (11j).** Product **11j** was prepared by the reaction of **9b** with $PhHgCBr_3$. Yield, 36%; mp 109-111 °C; IR (KBr): 3060, 3025 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.20 (d, J = 9.2 Hz, 1H, cyclopropyl H-3), 3.25 (d, J = 9.2 Hz, 1H, cyclopropyl H-2), 7.25-7.28 (m, 2H, 4-bromophenyl H-2, H-6), 7.29-7.40 (m, 2H, phenyl H-2, H-6), 7.33-7.43 (m, 3H, phenyl H-3, H-4, H-5), 7.52 (d, J = 8.5 Hz, 2H, 4-bromophenyl H-3, H-5). Anal. ($C_{15}H_{11}Br_3$): C, H.

***(E)*-1,1-Dibromo-2-(4-fluorophenyl)-3-phenylcyclopropane (11k).** Product **11k** was prepared by the reaction of **9c** with $PhHgCBr_3$. Yield, 31%; mp 59-61 °C; IR (KBr): 3050, 3032 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.23 (d, J = 9.4 Hz, 1H, cyclopropyl H-3), 3.26 (d, J = 9.4 Hz, 1H, cyclopropyl H-2), 7.08 (t, J = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.27-7.30 (m, 2H, phenyl H-2, H-6), 7.33-7.36 (m, 3H, phenyl H-3, H-4, H-5), 7.37 (dd, J_{HH} = 8.5 Hz, J_{HF} = 4.9 Hz, 2H, 4-fluorophenyl H-2, H-6); ^{19}F NMR ($CDCl_3$): δ 47.65 (dddd, J_{FCCH} = 8.5 Hz, J_{FCCCH} = 4.9 Hz, 1F). Anal. ($C_{15}H_{11}Br_2F.1/3H_2O$): C, H.

***(E)*-1,1-Dibromo-2-(4-methanesulfonylphenyl)-3-phenylcyclopropane (11l).** Product **11l** was prepared by reaction of **9e** with $PhHgCBr_3$. Yield, 59%; mp 138-140 °C; IR (KBr): 1320, 1152 (SO_2), 3046, 3026 (cyclopropyl C-H) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.10 (s, 3H, SO_2CH_3), 3.32 (d, J = 9.4 Hz, 1H, cyclopropyl H-3), 3.36 (d, J = 9.4 Hz, 1H, cyclopropyl H-2), 7.27-7.32 (m, 2H, phenyl H-2, H-6), 7.34-7.46 (m, 3H, phenyl H-3, H-4, H-5), 7.58 (d, J = 8.2 Hz, 2H, 4-methanesufonylphenyl H-2, H-6), 7.98 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 35.0 (cyclopropane C-3), 39.7 (cyclopropane C-2), 40.7 (cyclopropane C-1), 44.5 (SO_2CH_3), 127.4, 128.0, 128.2, 129.0 (phenyl *C*H

carbons, 4-methanesulfonylphenyl C-2, C-6), 130.0 (4-methanesulfonylphenyl C-3, C-5), 135.0 (phenyl C-1), 140.0 (4-methanesulfonylphenyl C-4), 142.1 (4-methanesulfonylphenyl C-1). Anal. ($C_{16}H_{14}Br_2O_2S$): C, H.

Analgesic Assay. Analgesic activity was determined using the 4% sodium chloride-induced writhing (abdominal constriction) assay [19,20] as described previously.

Antiinflammatory Assay. Antiinflammatory activity was determined using the carrageenan induced rat paw edema assay as reported previously.[21]

In Vitro Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) Inhibition Assays. The ability of the test compound to inhibit (IC_{50}, μM) the conversion of arachidonic acid (AA) to prostaglandin H_2 (PGH_2) by ram seminal vesicle cyclooxygenase-1 (*o*COX-1) and sheep placental cyclooxygense-2 (*o*COX-2) was determined using a COX-1/COX-2 inhibitor screening assay kit (Cayman Chemical, Ann Arbor, MI). Briefly, cyclooxygenase catalyzes the first step in the biosynthesis of AA to PGH_2. $PGF_{2\alpha}$ produced from PGH_2 by reduction with stannous chloride is measured by enzyme immunoassay (ACE™ competitive EIA). This assay is based on the competition between PGs and a PG-acetylcholinesterase conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells since the concentration of the PG tracer is held constant while the concentration of PGs varies. This antibody-PG complex binds to a mouse anti-rabbit monoclonal antibody that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's reagent, which contains the substrate to acetylcholinesterase, is added to the well. The product of this enzymatic reaction produces a distinct yellow color that absorbs at 405 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of PGs present in the well during the incubation: Absorbance $\propto$ [Bound PG Tracer] $\propto$ 1/PGs. Percent inhibition was calculated by comparison of compound-treated to various control incubations. The concentration of the test compound causing 50% inhibition (IC_{50}, μM) was calculated from the concentration-inhibition response curve (duplicate determinations). The validity of this in vitro COX-1/COX-2 inhibition assay was demonstrated using celecoxib (Celebrex®) as a reference drug where it gave values (COX-1 IC_{50} = 22.9 μM; COX-2 IC_{50} = 0.0567 μM; COX-2 selectivity index = 404) close to those previously reported.[4]

Molecular Modeling (Docking) Study. The coordinates from the X-ray crystal structure of murine COX-2 used in this simulation were obtained from the Protein Data Bank (PDB file 1cx2), where the active site is bound to the selective COX-2 inhibitor 4-[5-(4-bromophenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonamide (SC-558). The MM3 optimized structures of the *(E)*- (11h) and (*Z*)- (10h) stereoisomers of 1,1-dichloro-2-(4-

methanesulfonylphenyl)-3-phenylcyclopropane were subjected to dynamics optimization using the Alchemy 2000 program at 300K over a 0.001 ps time step for 1 ps. The lowest energy conformation obtained in this way was superimposed on SC-558 in the PDB file 1CX2 using the Insight II program, after which SC-558 was deleted. In order to relieve any unfavorable side chain overlaps and non bond-energies introduced in the model structure, the Measure/Bump command was used. Subsets of the enzyme were defined allowing residues within 10 Å of the ligand to relax, whereas all other enzyme residues were fixed. The Affinity command in the Docking module was used to complete the docking experiment. Minimization of the ligand-active site assembly was performed over 20,000 steps reaching a convergence of 0.01 Kcal/molÅ using the steepest descent method followed by the conjugate gradient method to reach a final convergence of 0.001 Kcal/molÅ. The CVFF force field was used in the docking experiment. The intermolecular energy of the drug-active site (assembly) interaction was used to evaluate the quality of the docking experiment.

Supporting Information on Elemental Analysis Data is Available in Appendix 1.1.

2.6.0.0. References

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Appendix 1.1:

Elemental Analysis for Compounds **8e, 10d, 10h-l, 11c, 11d** and **11e–l**.

Cmpd	Empirical Formula	Calculated			Found		
		C	H	N	C	H	N
8e	$C_{15}H_{14}O_2S$	69.74	5.46	–	69.41	5.13	–
10d	$C_{15}H_{11}Cl_2NO_2$	45.37	2.79	3.53	45.08	2.61	3.21
10h	$C_{16}H_{14}Cl_2O_2S.1/3H_2O$	55.29	4.16	–	55.28	4.10	–
10i	$C_{15}H_{11}Br_2Cl.1/3H_2O$	45.85	2.97	–	45.93	2.65	–
10j	$C_{15}H_{11}Br_3.1/2H_2O$	40.91	2.72	–	40.65	2.58	–
10k	$C_{15}H_{11}Br_2F.1/2H_2O$	47.48	3.16	–	47.25	2.90	–
10l	$C_{16}H_{14}Br_2O_2S.1/3H_2O$	44.02	3.36	–	44.04	3.02	–
11c	$C_{15}H_{11}Cl_2NO_2$	58.46	3.60	4.55	58.41	3.51	4.57
11d	$C_{15}H_{11}Br_2NO_2$	45.37	2.79	3.53	45.07	2.84	3.50
11e	$C_{15}H_{11}Cl_3.1/2H_2O$	58.70	3.91	–	58.50	3.52	–
11f	$C_{15}H_{11}BrCl_2.1/2H_2O$	51.27	3.41	–	51.02	3.11	–
11g	$C_{15}H_{11}Cl_2F.1/3H_2O$	62.68	4.06	–	63.04	3.87	–
11h	$C_{16}H_{14}Cl_2O_2S$	56.31	4.14	–	56.05	3.93	–
11i	$C_{15}H_{11}Br_2Cl$	46.60	2.87	–	46.43	2.74	–
11j	$C_{15}H_{11}Br_3$	41.81	2.57	–	42.03	2.47	–
11k	$C_{15}H_{11}Br_2F.1/3H_2O$	47.48	3.07	–	47.77	2.71	–
11l	$C_{16}H_{14}Br_2O_2S$	44.68	3.28	–	44.73	3.31	–

3.0.0.0. CHAPTER 2.0

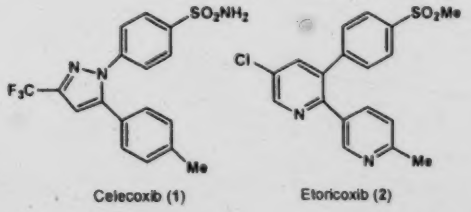
6-Alkyl, Alkoxy or Alkylthio-Substituted 3-(4-Methanesulfonylphenyl)-4-phenylpyran-2-ones: A Novel Class of Diarylheterocyclic Selective Cyclooxygenase-2 Inhibitors

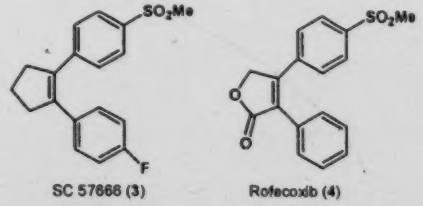
3.1.0.0. Introduction

Selective cyclooxygenase-2 (COX-2) inhibitors currently provide effective treatment of inflammatory disease states such as rheumatoid arthritis and osteoarthritis.[1] Recent studies have shown that selective COX-2 inhibitors can also induce apoptosis in colon, stomach, prostate and breast cancer cell lines.[2] Selective COX-2 inhibitors offer potential for the prophylactic prevention of inflammatory neurodegenerative disorders such as Alzheimer's disease.[3]

Diarylheterocycles constitute a major class of selective COX-2 inhibitors (Chart 2.1). In this regard, celecoxib (**1**) possesses a central 5-membered pyrazole ring, whereas etoricoxib (**2**) has a central 6-membered pyridine ring.[4] Extensive structural activity relationship (SAR) studies for the diarylheterocycle class have shown that a SO_2NH_2 or SO_2Me and F substituents at the *para*-position of one of the aryl rings often provides optimum COX-2 selectivity and potency.[5] Thus, the selective COX-2 inhibitor SC 57666 (**3**) has a sulfonylmethyl group at the *para* position of one phenyl ring along with a fluorine atom at the *para*-position on the other phenyl ring.[6] The highly selective COX-2 inhibitor rofecoxib (**4**) belongs to a diarylheterocylic class that possesses a central 5-membered lactone, [2-(5*H*)-furanone], ring system.[7] We describe herein the design, synthesis and biological evaluation of a novel class of diarylheterocyclic, 6-alkyl, alkoxy or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones, that possesses a central 6-membered lactone (pyran-2-one) ring.

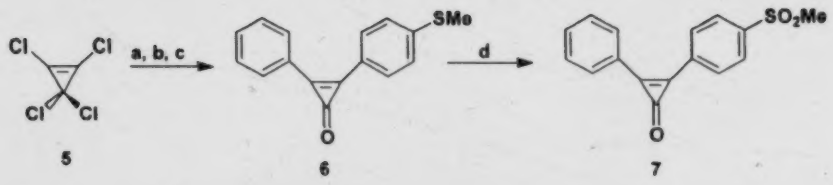
Chart 2.1: Representative examples of diarylheterocyclic selective COX-2 inhibitors





3.2.0.0. Chemistry

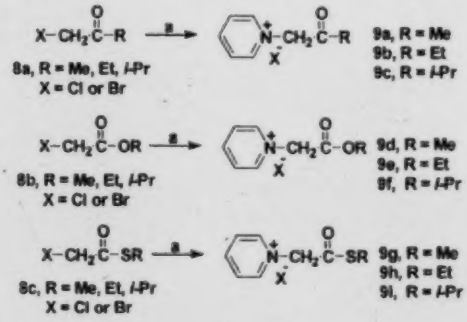
The synthetic reactions used for the synthesis of 6-alkyl, alkoxy or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**10a-c, 11a-c** and **12a-c**) are outlined in Schemes 2.1, 2.2 and 2.3. The 2,3-diphenylcyclopropenone (**6**) with a thiomethyl substituent at the *para*-position of one of the phenyl rings was prepared in moderate yields (22-



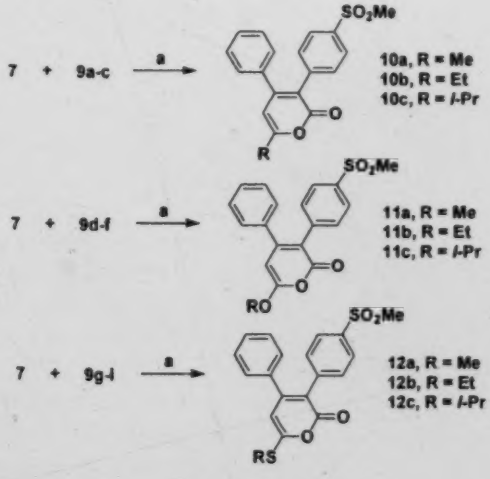
Scheme 2.1. Reagents and conditions: (a) dry $AlCl_3$, 1,2-dichloroethane, benzene, 25 °C, 24 h; (b) thioanisole, 25 °C, 24 h; (c) H_2O, 25 °C, 10 min; (d) aqueous Oxone®, THF- MeOH (1:1), 25 °C, 4-5 h.

33%) using a one-pot reaction starting with tetrachlorocyclopropene (**5**). The sequential arylation of **5** with benzene and methylthiobenzene, followed by hydration with ice-water yielded 2-(4-methylthiophenyl)-3-phenylcycloprop-2-ene-1-one (**6**) as the major product along with the 2-(2-methylthiophenyl)-3-phenylcycloprop-2-ene-1-one regioisomer as a minor product (ratio 4:1) which could not be purified by column chromatography. Subsequent oxidation of **6** using aqueous Oxone® solution afforded the methane sulfonyl product **7** as shown in Scheme 2.1.[8] The *N*-alkyl, alkoxy or alkylthiocarbonylmethyl pyridinium chlorides or bromides (**9a-i,** 33-68%) were prepared by the reaction of the respective alkyl, alkoxy or alkylthio-substituted chloro or bromoacetate (**8a, 8b** or **8c**) with pyridine as illustrated in Scheme 2.2.

The target 6-alkyl, alkoxy or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**10a-c, 11a-c** and **12a-c**) were prepared in low to moderate yields (9-44%) by the condensation of **7** with the respective pyridinium chlorides or bromides (**9a-c, 9d-f** or **9g-i**) in the presence of the base Et_3N to produce an intermediate ylide product that undergoes a ring expansion reaction to afford the title products (**10a-c, 11a-c** or **12a-c**) as illustrated in Scheme 2.3.[9] The structures



Scheme 2.2: Reagents and conditions: (a) pyridine, THF, 25 °C, 6 h.



Scheme 2.3: Reagents and conditions: (a) benzene, triethylamine, 25 °C, 16-18 h.

of **10-12** were confirmed by microanalyses data and ^{1}H NMR NOE studies which showed NOE interactions between H-5 and the 6-alkyl (R), alkoxy (O-R) or alkylthio (S-R) moiety, and between H-5 and the C-4 ortho-phenyl hydrogens, which establishes the regiochemistry of the C-4 and C-5 phenyl rings.

3.3.0.0. Results and discussion

The effect of the C-6 alkyl (**10a-c**), alkoxy (**11a-c**) and alkylthio (**12a-c**) substituents on the central 6-membered lactone (pyran-2-one) ring on COX-2 selectivity and potency was determined by in vitro COX-1/COX-2 inhibition studies. The structure activity relationships acquired showed that this lactone class of compounds are moderate to potent selective COX-2 inhibitors (see data in Table 2.1).

The 6-alkyl-3-(4-methanesulfonyl phenyl)-4-phenylpyran-2-ones (**10a-c**), show weak to moderate COX-1 inhibition (8.0-614.8 μM range) with good COX-2 inhibition in the 0.5-1.5 μM range. In the alkoxy series (**11a-c**), good COX-2 inhibitory activity and selectivity was shown by the 6-ethoxy derivative **11b** (COX-1 IC_{50} = 281.5 μM; COX-2 IC_{50} = 1.3 μM; COX-2 Selectivity Index = 216.5). Introduction of a thioethyl (EtS-) substituent at C-6 of the central pyran-2-one ring led to a dramatic increase in COX-2 selectivity and potency, with **12b** showing a weak COX-1 inhibition (COX-1 IC_{50} = 386.2 μM) and potent inhibition of COX-2 (COX-2 IC_{50} = 0.0032 μM) for a very high COX-2 S.I. > 120,000 relative to the reference drug rofecoxib (COX-2 IC_{50} = 0.4279; S.I. > 1,168).

The critical difference between the binding sites for COX-1 and COX-2 is at position 523 where COX-2 has the amino acid residue Val in place of the bulkier Ile in COX-1. This difference produces a secondary pocket extending off the primary binding site in COX-2 that is absent in COX-1. Consequently, the combined volume of the primary binding site and the secondary pocket in COX-2 is about 25% larger (394 Å^3) than the volume of the COX-1 binding site (316 Å^3).[10] This difference in volume can be exploited to manipulate COX-2 selectivity of diarylheterocyclic classes of COX-2 inhibitors, by varying the volume of the drug and the appropriate placement of substituents with varying electronic and steric properties.[11]

It is well established for the diarylheterocyclic class of COX-2

Table 2.1: In vitro inhibition of COX-1 and COX-2 by 6-alkyl, 6-alkoxy or 6-alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**10a-c, 11a-c** and **12a-c**).

Compd	COX-1 inhibition IC_{50}, μM[a]	COX-2 inhibition IC_{50}, μM[a]	COX-2 S.I.[b]	Volume (Å^3)[c]
10a	614.8	0.68	904.0	294.0
10b	8.0	1.5	5.0	310.0
10c	341.5	0.50	683.0	327.0
11a	14.7	28.3	---	302.0
11b	281.5	1.3	216.5	320.0
11c	4.0	2.0	2.0	336.0
12a	> 100	2.8	36.0	311.0
12b	386.2	0.0032	120,000	329.0
12c	> 100	> 100	---	346.0
Rofecoxib	> 500	0.4279	> 1,168	267.0
Celecoxib	22.9	0.0567	404	298.0

[a] Values are means of two determinations acquired using an ovine COX-1/COX-2 assay kit (Catalog No. 560101, Cayman Chemicals Inc., AnnArbor, MI) and the deviation from the mean is < 10% of the mean value.

[b] In vitro COX-2 selectivity index (IC_{50} COX-1/IC_{50} COX-2).

[c] The volume of the molecule, after minimization using the MM3 forcefield, was calculated using the Alchemy 2000 program.

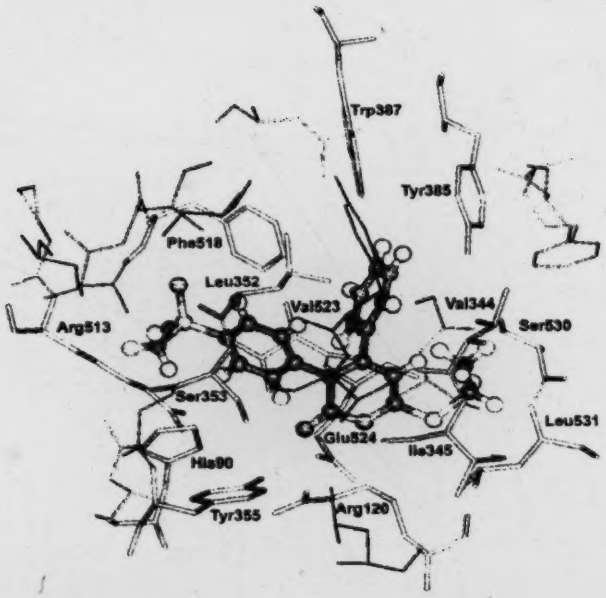


Figure 2.1. Docking of **12b** (ball and stick) in the active site of murine COX-2 (line and stick) ($E_{intermolecular}$ = -90.81 kcal/mol). Hydrogen atoms of the amino acid residues are removed to increase clarity.

inhibitors, that a *para*-methylsulfone or sulfonamide substituent on one of the phenyl rings is a requirement for good COX-2 potency and selectivity.[5] Accordingly, the 6-alkyl, 6-alkoxy and 6-alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one group of compounds were designed to have a -SO_2Me substituent at the *para*-position of one of the phenyl rings. Compounds **10a-c, 11a-c** and **12a-c** have volumes in the range of 293-345 Å^3, relative to the selective COX-2 inhibitor rofecoxib (267.0 Å^3) as shown in Table 2.1. In general, for this series of compounds, COX-2 selectivity and potency was dependant upon steric and electronic properties of the C-6 substituent on the central pyran-2-one ring which positions sulfonylmethyl moiety in the vicinity of the secondary pocket of COX-2.

The orientation of the highly potent and selective COX-2 inhibitor, 6-ethylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (**12b**), in the COX-2 active site was examined by a docking experiment (Fig. 2.1).[12] This study showed that **12b** binds in the center of the primary binding site of COX-2 with the SO_2Me moiety interacting with the secondary pocket amino acid residues (Phe^{518}, Gln^{192}, Arg^{513}, Leu^{352}, Ser^{353} and Val^{523}). One of the *O*-atoms of the SO_2Me substituent forms a hydrogen bond with the amide hydrogen of Phe^{518} (1.92 Å). The ring *O*-atom of the central lactone (pyran-2-one) is oriented in the direction of the polar amino acid Arg^{120} at the mouth of the channel, where this *O*-atom is about 4.24 Å away from the NH_2 (guanidino) group. The C=*O* of the central pyran-2-one is hydrogen bonding with the O*H* of Tyr^{355} (1.70 Å). These interactions may disrupt the salt bridge

between His^{90}, Arg^{120}, Tyr^{355} and Glu^{524} at the mouth of the COX-2 active site. The unsubstituted phenyl ring lies in a hydrophobic cavity lined by Tyr^{385}, Trp^{387}, Tyr^{348} and Ser^{530}. Interestingly, the C-6 EtS-substituent is located in a hydrophobic region formed by Val^{344}, Ile^{345}, Val^{349}, Ser^{530} and Leu^{531}, with the *S*-atom forming a weak hydrogen bond with the O*H* of Ser^{530} (5.08 Å). This shows the importance of C-6 substituent in orienting the molecule such that the methylsulfone moiety inserts into the secondary pocket of COX-2. A similar docking study for the less potent, and less selective, COX-2 inhibitory C-6 OEt analogue (**11b**) showed that the SO_2Me moiety is inserted less deeply into the secondary pocket than the C-6 SEt of **12b**, the lactone ring oxygen atom in **11b** is closer to the NH_2 of Arg^{120} (3.26 Å) relative to 4.24 Å in **12b**, that the C-6 OEt oxygen atom is not within hydrogen bonding distance of the O*H* of Ser^{530} (6.63 Å), and the intermolecular energy for the ligand-enzyme complex for **11b** is higher (–87.60 kcal/mol). These observations together with the larger volume (329.0 $Å^3$), provides a good explanation for the potent and selective inhibitory activity of **12b**.

3.4.0.0. Conclusions

The results of this investigation show (i) a C-6 thioethyl (-SEt) substituent (**12b**)[13] in this 3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one class of diarylheterocycles provides potent and selective inhibition of the COX-2 isozyme, (ii) molecular modeling studies indicate the SO_2Me moiety inserts deep into the COX-2 secondary pocket and the C-6 *S*Et sulfur atom forms a weak hydrogen bond with the O*H* atom of Ser^{530} and (iii) these C-6 alkyl, alkoxy and alkylthio compounds **10-12** could serve as useful probes to study the function and catalytic activity of the COX-2 isozyme.

3.5.0.0. References and Notes

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(12) Docking studies were performed using Insight II software Version 2000.1 (Accelrys Inc.) running on a Silicon Graphics Octane 2 R14000A workstation. The coordinates of the X-ray crystal structure of the selective COX-2 inhibitor SC-558 bound to the murine COX-2 enzyme was obtained from the RCSB Protein Data Bank (1cx2) and hydrogens were added. The ligand molecules were constructed using the Builder module and were energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The energy minimized ligands were superimposed on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. The resulting structure (ligand-enzyme assembly) was minimized using the Discover module for 5000 iterations or until an RMSD of 0.05 Å was reached using consistent valence force field (CVFF). Further optimization of the ligand-enzyme complex was obtained using the Affinity command in the Docking module of Insight II by defining subsets of the enzyme such that residues within 10 Å of the ligand were allowed to relax, while the rest of the enzyme residues were fixed. The optimal binding orientation was achieved by utilizing 300

steps of steepest descent followed by the conjugate gradient method. The CVFF was employed for all docking purposes. These docked structures were very similar to the minimized structures obtained initially. The quality of the docked structures were evaluated by measuring the intermolecular energy of the ligand-enzyme assembly.

(13) Analytical data for **12b**. Mp 175–176 °C; IR (KBr): 1718 (C=O), 1314, 1153 (SO_2) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 1.46 (3H, t, J = 7.3 Hz, SCH_2CH_3), 3.03 (3H, s, SO_2CH_3), 3.15 (2H, q, J = 7.3 Hz, SCH_2CH_3), 6.35 (1H, s, pyranone H-5), 7.04-7.07 (2H, m, phenyl H-2, H-6), 7.21-7.31 (3H, m, phenyl H-3, H-4, H-5), 7.35 (2H, d, J = 8.5 Hz, 4-$MeSO_2$-C_6H_4 H-2, H-6), 7.78 (2H, d, J = 8.5 Hz, $MeSO_2$-C_6H_4 H-3, H-5). Anal. Calcd for $C_{20}H_{18}O_4S_2$: C 62.16, H 4.69. Found: C 62.04, H 4.85.

4.0.0.0. CHAPTER 3.0

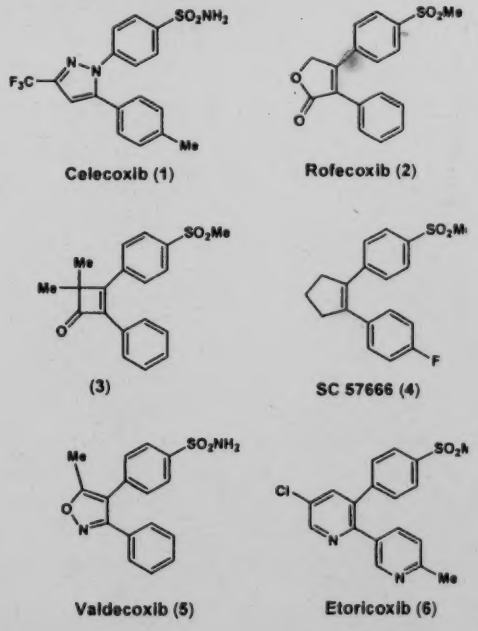
Design, Synthesis and Biological Evaluation of 6-Substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones: A Novel Class of Diarylheterocyclic Selective Cyclooxygenase-2 Inhibitors

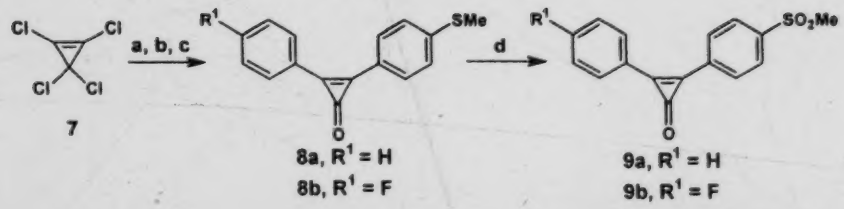
4.1.0.0. Introduction

The differential tissue distribution of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) provides a rationale for the development of selective COX-2 inhibitors as antiinflammatory-analgesic agents that lack the GI side effects exhibited by traditional nonsteroidal-antiinflammatory drugs (NSAIDs).[1-4] This hypothesis has been applied successfully in the design of two highly selective tricyclic COX-2 inhibitors that possess a diaryl heterocyclic ring template, namely celecoxib (**1**), and rofecoxib (**2**) respectively (Chart 1).[5,6] In addition to its role in rheumatoid arthritis and osteoarthritis, COX-2 is also implicated in colon cancer and angiogenesis.[7–9] Recent studies have shown that the progression of Alzheimer's disease is reduced among some users of NSAIDs. Chronic treatment with selective COX-2 inhibitors may therefore slow the progress of Alzheimer's disease, without causing GI damage.[10]

Diarylheterocycles, and other central ring pharmacophore templates, have been extensively studied as cyclooxygenase inhibitors. All these tricyclic molecules possess 1,2-diaryl substitution on a central 4-, 5- or 6-membered ring system such as cyclobutenone (**3**), cyclopentene (**4**), isoxazole (**5**), pyrazole (**1**), 2-(5*H*)-furanone (**2**) or pyridine (**6**), respectively (Chart 3.1).[5, 11-15] Structure-activity relationship (SAR) studies have shown that for optimum COX-2 selectivity and potency, a SO_2Me or SO_2NH_2 substituent at the *para*-position of a phenyl ring, and that the presence of a *para*-F substituent on the non-sulfonyl vicinal phenyl ring improves in vivo activity.[16] In a very recent letter, we reported the design and synthesis of a novel class of diarylheterocycle with a central 6-membered lactone (pyran-2-one) ring which exhibited good in vitro COX-2 inhibitory potency and selectivity.[17] The Merck Co. has also developed novel methods to synthesize diarylheterocycles

Chart 3.1: Representative Examples of Selective Tricyclic COX-2 Inhibitors



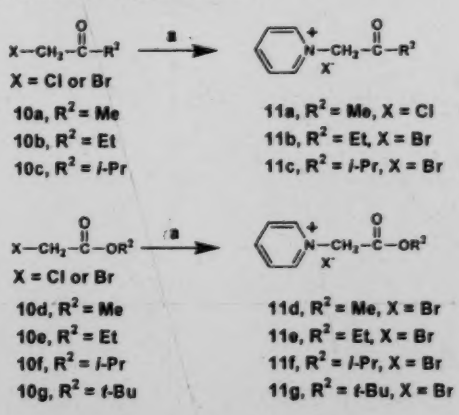


Scheme 3.1: Reagents and conditions: (a) dry $AlCl_3$, 1,2-dichloroethane, R^1-C_6H_5 (R^1 = H, F), 25 °C, 24 h; (b) thioanisole, 25 °C, 24 h; (c) H_2O, 25 °C, 10 min; (d) aqueous Oxone®, THF, MeOH, 25 °C, 4–5 h.

having a central 6- membered lactone (pyran-2-one) ring system.[18] We now describe the design, synthesis and biological evaluation of a diverse group of 6-alkyl-, 6-alkoxy- or 6-alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones as selective COX-2 inhibitors with antiinflammatory-analgesic activities.

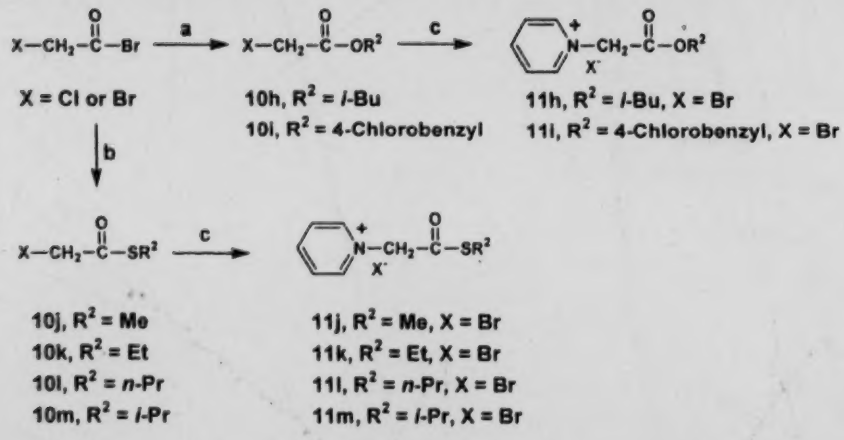
4.2.0.0. Chemistry

The title 3,4-diphenylpyran-2-ones (**14a–v**), possessing a central 6-membered lactone ring with either a 6-alkyl, alkoxy, or alkylthio substituent, were prepared by the condensation of a substituted 2,3-diphenylcycloprop-2-en-1-one (**9a** or **9b**) with the respective pyridinium ylide derivative of **11a-m** as illustrated in Schemes 3.1, 3.2, 3.3 and 3.4. The 2-(4-methanesulfonylphenyl)-3-phenylcycloprop-2-en-1-one (**9a**), and the 3-(4-fluorophenyl) analog (**9b**), were prepared using a one-pot reaction starting with tetrachlorocyclopropene **7** according to our previously reported procedure as shown in Scheme 3.1.[19] The pyridinium salts **11a–g** were prepared by the reaction of the respective haloalkylketone (**10a–c**), or alkyl haloacetate (**10d–g**) with pyridine (Scheme 3.2). The nucleophilic substitution reaction of either chloroacetyl chloride or bromoacetyl bromide with various alcohols, or thiols, gave the respective alkyl haloacetate (**10h–i**), or alkyl halothioacetate (**10j–m**), (which on subsequent reaction with pyridine afforded the respective *N*-alkoxy, or alkylthiocarbonyl methylpyridinium halides (**11h–m**) in moderate to good yields (33–68%) as shown in Scheme 3.3.

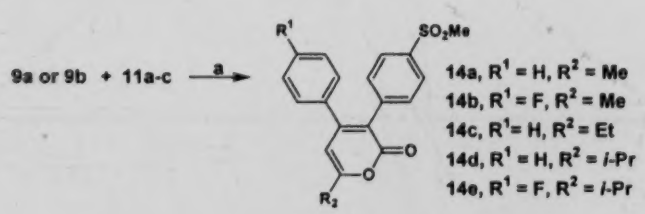

X = Cl or Br
10a, R2 = Me
10b, R2 = Et
10c, R2 = i-Pr
11a, R2 = Me, X = Cl
11b, R2 = Et, X = Br
11c, R2 = i-Pr, X = Br
X = Cl or Br
10d, R2 = Me
10e, R2 = Et
10f, R2 = i-Pr
10g, R2 = t-Bu
11d, R2 = Me, X = Br
11e, R2 = Et, X = Br
11f, R2 = i-Pr, X = Br
11g, R2 = t-Bu, X = Br

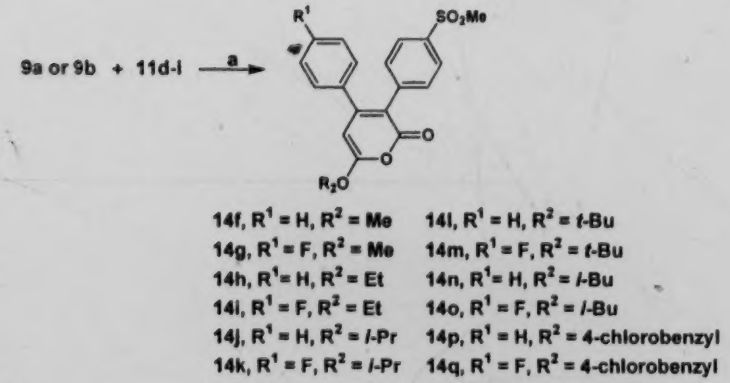
Scheme 3.2: Reagents and conditions: (a) pyridine, THF, 25 °C, 6 h.

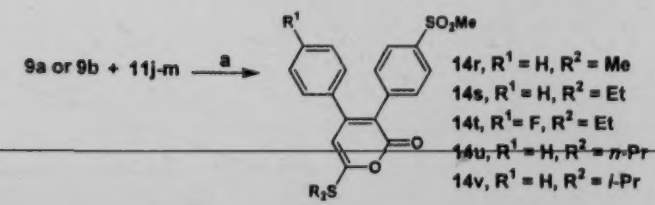
The 3,4-diarylpyran-2-ones (**14a–v**) were prepared in low to moderate yields (8–44%) by condensation of a 2-(4-methanesulfonylphenyl)-3-phenylcycloprop-2-en-1-one (**9a** or **9b**) with a pyridinium salt (**11a–m**) in the presence of the base triethylamine. The initial ylide compound that is formed in the reaction undergoes a spontaneous


X = Cl or Br
10h, R² = i-Bu
10i, R² = 4-Chlorobenzyl
11h, R² = i-Bu, X = Br
11i, R² = 4-Chlorobenzyl, X = Br
10j, R² = Me
10k, R² = Et
10l, R² = n-Pr
10m, R² = i-Pr
11j, R² = Me, X = Br
11k, R² = Et, X = Br
11l, R² = n-Pr, X = Br
11m, R² = i-Pr, X = Br

Scheme 3.3: Reagents and conditions: (a) diethyl ether, R^2OH (R^2 = *i*-Bu or 4-Cl-C_6H_4-CH_2), -23 °C → 25 °C, 4 h; (b) diethyl ether, R^2SH or $R^2S^-Na^+$ (R^2 = Me, Et, *n*-Pr or *i*-Pr), -23 °C → 25 °C, 4 h; (c) pyridine, THF, 25 °C, 6 h.

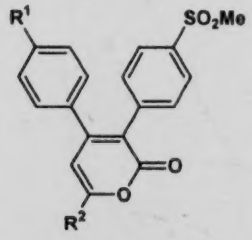



9a or 9b + 11d-l
14f, R¹ = H, R² = Me
14g, R¹ = F, R² = Me
14h, R¹ = H, R² = Et
14i, R¹ = F, R² = Et
14j, R¹ = H, R² = i-Pr
14k, R¹ = F, R² = i-Pr
14l, R¹ = H, R² = t-Bu
14m, R¹ = F, R² = t-Bu
14n, R¹ = H, R² = i-Bu
14o, R¹ = F, R² = i-Bu
14p, R¹ = H, R² = 4-chlorobenzyl
14q, R¹ = F, R² = 4-chlorobenzyl

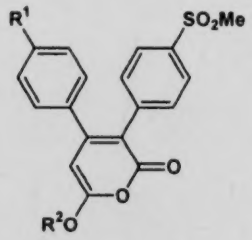


Scheme 3.4: Reagents and conditions: (a) benzene, Et_3N, 25 °C, 16-18 h.

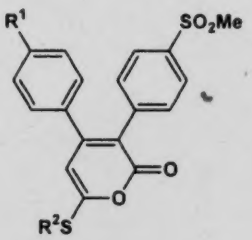
Table 1. COX-1/COX-2 Inhibitory and Antiinflammatory-Analgesic Activities of 6-Alkyl-, 6-Alkoxy-, and 6-Alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**14a–v**).



14a-e



14f-q



14r-v

Cmpd	R^1	R^2	IC_{50} (μM) [a] COX-1	IC_{50} (μM) [a] COX-2	Selectivity Index [b]	AI Activity [c] % Inhibition at 3 hours	AI Activity [c] % Inhibition at 5 hours	Analgesic Activity [d] % Inhibition at 30 min.	Analgesic Activity [d] % Inhibition at 60 min.	Volume ($Å^3$) [e]
14a	H	Me	614.8	0.68	904.0	12.5 ± 2.2	23.5 ± 3.2	57.0 ± 4.1	60.4 ± 7.3	293.5
14b	F	Me	201.5	125.3	1.6	–	–	–	–	296.6
14c	H	Et	8.0	1.5	5.3	–	–	–	–	310.1
14d	H	*i*-Pr	341.5	0.50	683.0	09.8 ± 3.1	19.5 ± 5.3	67.8 ± 9.1	56.9 ± 7.1	326.7
14e	F	*i*-Pr	15.7	13.6	1.1	35.8 ± 4.8	69.1 ± 4.9	50.9 ± 4.2	20.7 ± 5.4	329.8
14f	H	Me	14.7	28.3	< 0.5	Inactive	22.0 ± 5.4	55.2 ± 10.1	83.4 ± 1.9	301.7
14g	F	Me	> 1000	59.5	> 20	30.6 ± 4.4	30.0 ± 6.5	49.0 ± 10.5	72.6 ± 4.6	305.5
14h	H	Et	281.5	1.3	216.5	–	–	–	–	318.7
14i	F	Et	288.0	0.10	2880	32.7 ± 5.3	67.8 ± 5.5	42.8 ± 8.2	75.7 ± 4.1	322.3
14j	H	*i*-Pr	4.0	2.0	2.0	–	–	–	–	336.0
14k	F	*i*-Pr	560.0	83.6	6.7	–	–	–	–	338.7
14l	H	*t*-Bu	> 100	2.7	> 37	29.0 ± 2.8 [f]	30.1 ± 3.0 [f]	73.8 ± 4.0 [g]	80.9 ± 6.2 [g]	351.3
14m	F	*t*-Bu	68.5	20.4	3.3	–	–	–	–	355.6
14n	H	*i*-Bu	3.1	1.8	> 1.7	–	–	–	–	351.7
14o	F	*i*-Bu	42.6	35.0	1.2	–	–	–	–	355.5
14p	H	4-Cl-$PhCH_2$	50.7	415.8	< 0.12	–	–	–	–	386.5
14q	F	4-Cl-$PhCH_2$	301.5	53.3	5.6	–	–	–	–	390.8
14r	H	Me	> 100	2.8	> 35.7	–	–	–	–	311.5
14s	H	Et	386.2	0.0032	> 120,687	09.2 ± 3.0 [f]	19.5 ± 2.4 [f]	81.0 ± 5.4 [g]	77.4 ± 8.1 [g]	328.5
14t	F	Et	> 100	1.44	> 69.4	25.8 ± 4.0 [f]	16.8 ± 7.1 [f]	54.3 ± 11.5 [g]	66.0 ± 6.5 [g]	331.2
14u	H	*n*-Pr	> 100	> 100	–	–	–	–	–	344.6
14v	H	*i*-Pr	> 100	> 100	–	–	–	–	–	345.6
Ibuprofen						56.2 ± 2.0 [f]	41.5 ± 4.9 [f]	–	–	209.5
Celecoxib			22.9	0.057	> 401	79.9 ± 1.9 [f,h]	58.2 ± 1.8 [f]	31.7 ± 9.6 [g]	62.0 ± 7.3 [g]	298.5
Rofecoxib			> 500	0.43	> 1,162	–	–	–	–	267.2

[a] Values are means of two determinations and deviation from the mean is < 10% of the mean value. [b] In vitro COX-2 selectivity index (IC_{50} COX-1/IC_{50} COX-2). [c] Inhibitory activity on carrageenan-induced rat paw edema. The results are expressed as mean ± SEM (n = 4–6) following a 1mg/kg oral dose of the test compound. [d] Inhibitory activity in the rat 4% NaCl-induced abdominal constriction assay. The results are expressed as mean ± SEM (n = 4–6) following a 1mg/kg intraperitoneal dose of the test compound. [e] The volume of the molecule after minimization using the PM3 forcefield, was calculated using the Alchemy 2000 program. [f] 50 mg/kg po dose. [g] 50 mg/kg ip dose. [h] ID_{50} = 10.8 mg/kg po dose.

ring expansion[20-22] reaction to produce the target 6-alkyl-, 6-alkoxy- or 6-alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**14a–v**) having a central 6-membered lactone ring (pyran-2-one) as illustrated in Scheme 3.4. No other regioisomer was observed using the reaction conditions employed. The structures of compounds **14** were consistent with microanalytical and mass spectral data. ^{1}H NMR nuclear Overhauser enhancement (nOe) studies showed nOe interactions between H-5 and the 6-alkyl (R^2), alkoxy (OR^2) oralkylthio (SR^2) moiety, and between H-5 and the C-4 *ortho*-phenyl hydrogens, which establishes the regiochemistry of the C-4 and C-5 phenyl rings.

4.3.0.0. Results and Discussion

The in vitro abilities (IC_{50} values) of the title compounds (**14a–v**) to inhibit the isozymes COX-1 and COX-2 were determined by modification of a previously reported procedure (Table 1).[23] The rational design of selective COX-2 inhibitors frequently exploits the difference in the volume of the binding sites for COX-1 and COX-2. Thus a SO_2Me or a SO_2NH_2 substituent placed at the *para*-position of one of the phenyl rings can interact with secondary pocket amino acid residues such as His[90], Gln[192], Phe[518] and Arg[513] that are associated with, and accessible from, the primary COX-2 binding site.[24,25] Accordingly, we have designed a novel class of 6-substituted-3,4-diphenylpyran-2-ones having a C-3 4-(methanesulfonylphenyl) substituent in conjunction with either a C-4 phenyl, or 4-fluorophenyl, substituent.

In vitro enzyme inhibition studies for the C-6 alkyl (Me, Et, *i*-Pr) subgroup of compounds (**14a–e**) showed weak to good COX-2 inhibitory activity (IC_{50} values in the 0.5–125.3 μM range) with 6-methyl-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (**14a**), showing the best combination of COX-2 inhibitory potency and selectivity index (IC_{50} = 0.68 μM; S.I. = 904).

In this C-6 alkyl subgroup (**14a–e**) of compounds, a *para*-fluoro substituent on the C-4 phenyl ring decreased both COX-2 selectivity and inhibitory potency but increased COX-1 inhibitory potency (Table 1). In the C-6 alkoxy (OMe, OEt, OPr-*i*, OBu-*t*, OBu-*i*, OCH_2-C_6H_4-4-Cl) subgroup of compounds **14** having a C-4 phenyl substituent, in vitro COX-2 inhibitory activity may not be totally dependent upon steric effects even though the C-6 OEt, *i*-PrO, *t*-BuO and *i*-BuO analogs were equipotent (IC_{50} values in the 1.3 to 2.7 μM range). Introduction of a C-6 4-chlorobenzyloxy substituent (**14p**) significantly reduced COX-2 inhibitory activity and selectivity (COX-2 IC_{50} = 415.8 μM; COX-1 IC_{50} = 50.7 μM; S.I. < 0.12) as shown in Table 3.1. Compounds having a C-4 4-fluorophenyl substituent are generally less potent COX-2 inhibitors than the corresponding C-4 phenyl analogs, except for the C-6 OEt compound **14i** (COX-2 IC_{50} = 0.10 μM; COX-1 IC_{50} = 288 μM; S.I. = 2880). The C-6 substituent in the thioalkyl (MeS, EtS, *n*-PrS, *i*-PrS) subgroup of compounds had a significant effect on COX-2 inhibitory potency (EtS >> MeS > inactive *n*-PrS and *i*-PrS) and COX-2 selectivity. For example, the C-6 thioethyl compound **14s** is a highly potent and selective COX-2 inhibitor (COX-1 IC_{50} = 386.2 μM; COX-2 IC_{50} = 0.0032 μM; S.I. > 120,000) relative to the reference drugs rofecoxib (COX-2 IC_{50} = 0.43 μM; S.I. > 1,162) and celecoxib (COX-2 IC_{50} =

0.057 μM; S.I. > 401). The in vitro cyclooxygenase inhibition data for this series (**14a–v**) of compounds illustrate the flexibility of the COX-2 active site in accommodating compounds with a range of molecular volumes (293–390 Å^3).

In an earlier communication, we attributed the excellent in vitro COX-2 inhibitory activity exhibited by **14s** to the presence of a weak hydrogen bonding interaction of the sulfur atom of the C-6 thioethyl (-SEt) substituent with the O*H* of Ser^{530} in the COX-2 binding site.[17] A recent molecular modeling study has confirmed the importance of Ser^{530} in the COX-2 inhibitory potency of diarylheterocyclic COX-2 inhibitors.[26] In order to explain the potent COX-2 (IC_{50} = 0.10 μM), and weak COX-1 inhibitory (IC_{50} = 288 μM), activity of **14i** [6-ethoxy-3-(4-methanesulfonyl phenyl)-4-(4-fluorophenyl)pyran-2-one], a molecular modeling study was performed where **14i** was docked in the binding sites of both COX-1 and COX-2. In addition, molecular dynamics (MD) simulations were carried out to assess the stability of the docked ligand–enzyme complexes. Docking **14i** in the COX-2 active site shows that it binds in the primary binding site such that the *p*-SO_2Me substituent orients towards the secondary pocket amino acid residues (His^{90}, Gln^{192}, Arg^{513}, Phe^{518}, Val^{523} and Leu^{352}) with one of the *O*-atoms of the SO_2Me substituent forming a hydrogen bond with the amide hydrogen of Phe^{518} (2.1 Å) as shown in Figure 3.1. The C=*O* oxygen atom of the central pyran-2-one ring is oriented towards Tyr^{355} which is part of the entrance to the secondary pocket. This C=*O* oxygen atom is positioned about 3.64 Å from the O*H* of Tyr^{355} and the *O*-atom of the central pyran-2-one ring is about 4.7 Å from the NH_2 (guanidino group) of Arg^{120}.

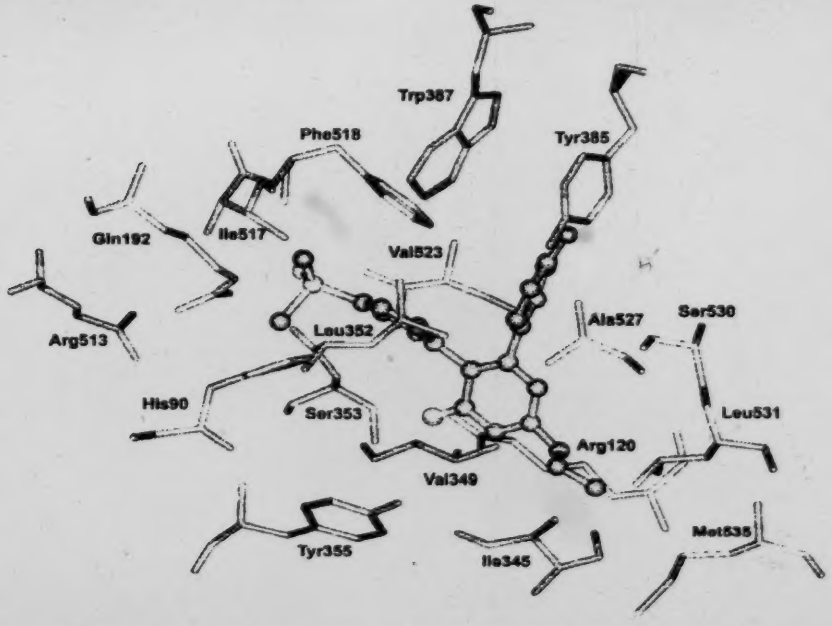


Figure 3.1: Docking 6-ethoxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (**14i**) (ball and stick) in the active site of murine COX-2 ($E_{intermolecular}$ = -60.7 kcal/mol). Hydrogens atoms are not shown for clarity.

The C-4 substituted phenyl ring lies in a hydrophobic cavity lined by Tyr^{385} and Trp^{387} with the *p*-fluoro substituent located about 5.8 Å away from the O*H* of Tyr^{385}, and where the distance between the center of the C-4 phenyl ring and the *O*H of Ser^{530} was about 6.9 Å. A recent study has shown the importance of Ser^{530} in the COX-2 inhibitory activity of rofecoxib.[26] Accordingly, the C-6 ethoxy (-OEt) substituent of **14i** is located in a hydrophobic region formed by Val^{349}, Ile^{345}, Ser^{530}, Leu^{531} and Met^{535} with the *O*-atom of C-6 OEt substituent positioned about 4.9 Å away from the O*H* of Ser^{530} (Figure 3.1).

Similar docking of **14i** on the COX-1 active site showed the drug–enzyme binding interaction was not dramatically different from that observed in COX-2 (Figure 3.2). However, closer examination revealed potentially crucial interactions involving the central pyran-2-one C=*O* with the key active site amino acid residue Tyr^{355}, the interaction of the C-3 *para*-SO_2Me substituent with Ile/Val^{523} that forms part of the entrance to the secondary pocket and interaction of the C-6 OEt substituent with Ser^{530} that may contribute to the in vitro COX-2 inhibitory activity of **14i**. The importance of Tyr^{355} in the binding affinities of diarylheterocyclic COX-2 inhibitors was shown in a recent study, wherein mutation of Tyr^{355} to Ala in the COX-2 active site lead to increased dissociation rates of celecoxib and valdecoxib.[29] Docking **14i** in the COX-1 active site suggests that the C=*O* oxygen atom is separated by a greater distance from the O*H* of Tyr^{355} (5.0 Å) than observed for a similar interaction in COX-2 (3.64 Å), and that insertion of the *p*-SO_2Me substituent into the secondary pocket is

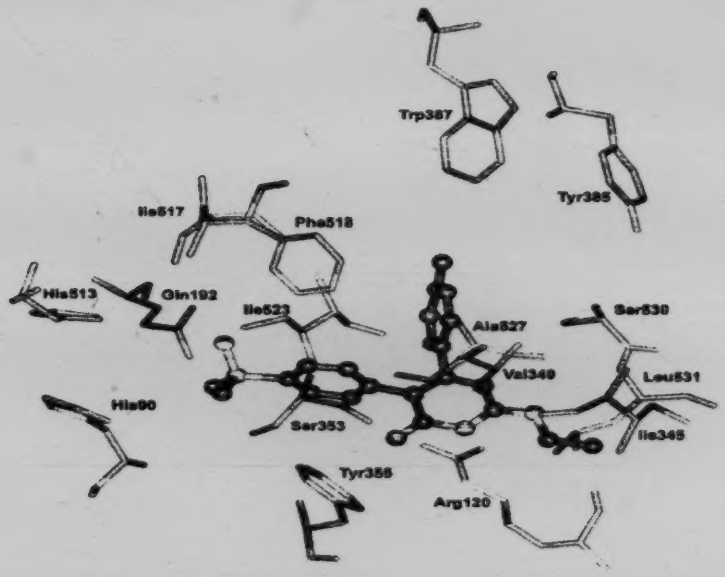


Figure 3.2: Docking 6-ethoxy-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (**14i**) (ball and stick) in the active site of ovine COX-1 ($E_{intermolecular}$ = -49.3 kcal/mol). Hydrogens atoms are not shown for clarity.

sterically impeded by the bulky Ile[523] as shown in Figure 3.2. In addition, the C-6 ethoxy oxygen atom is further removed (5.5 Å) from the O*H* of Ser[530] compared to COX-2 (4.9 Å). It has been shown that a single amino acid difference (Ile/Val) at position 523 in the respective active sites of COX-1 and COX-2 can be exploited to design diarylheterocyclic COX-2 inhibitors.[24,25] However, a recent molecular modeling study of the diarylheterocyclic COX-2 inhibitor rofecoxib in the COX-1 active site has shown that steric hindrance due to the substitution of Ile/Val[523] is not enough to prevent binding of the ligand to COX-1.[27] Recent studies have also shown that COX-2 inhibitors belonging to the diarylheterocyclic class show time dependent inhibition of COX-2 while exhibiting, competitive and reversible inhibition of COX-1.[28,29] Consequently, the higher binding affinity exhibited by **14i** for COX-2 may be due in part to its slow dissociation from COX-2. This possibility is consistent with results from a molecular dynamics simulation of **14i** docked in the active site of COX-2 which showed a lower intermolecular energy ($E_{intermolecular}$ = -60.7 kcal/mol) for this ligand–enzyme complex as compared to **14i** docked in the active site of COX-1 ($E_{intermolecular}$ = -49.3 kcal/mol).

In vivo pharmacological evaluation of some title compounds **14** was carried out to assess their potential antiinflammatory and analgesic activities. Initial compound selection for in vivo screening was based on in vitro COX-1/COX-2 enzyme inhibition data obtained. Qualitative structure–activity relationship data, acquired using the antiinflammatory rat paw edema assay showed that this group of C-6 alkyl-, alkoxy-, or alkylthio substituted 3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones exhibit antiinflammatory activity in the inactive to good activity range (inactive–69% inhibition) (Table 3.1). In the C-6 alkyl series (**14a**, **14d** and **14e**), 6-isopropyl-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (**14e**) was the most active antiinflammatory agent (35 and 69% reduction in inflammation at 3 and 5 h post-drug administration, respectively) for a 1 mg/kg oral dose as compared to reference drug celecoxib (79 and 58% reduction in inflammation at 3 and 5 h post-drug administration respectively) for a 50 mg/kg oral dose.

The C-6 alkoxy substituted group of compounds (**14f, 14g, 14i** and **14l**) reduced inflammation by 0–67% at different time intervals as shown in Table 3.1. 6-Ethoxy-3-(4-methanesulfonyl phenyl)-4-(4-fluorophenyl)pyran-2-one (**14i**) was the most potent antiinflammatory agent in this series producing a 32 and 67% reduction in inflammation at 3 and 5 h post-drug administration respectively for a 1 mg/kg oral dose. This in vivo antiinflammatory activity parallels its in vitro COX-2 potency and selectivity (COX-2 IC_{50} = 0.10 μM; S.I. = 2880). In general, the C-6 alkyl and alkoxy substituted compounds, which possess a *para*-fluoro substituent on the C-4 phenyl ring, exhibit good antiinflammatory activity (Table 3.1). In contrast, the C-6 thioethyl compound **14s**, which exhibited excellent in vitro COX-2 potency and selectivity (IC_{50} = 0.0032 μM; S.I. > 120,000), was a weak antiinflammatory agent (9 and 19.5% reduction in inflammation at 3 and 5 h post-drug administration, respectively) for a 50 mg/kg oral dose. When **14s** was administered at lower doses (1, 5 and 10 mg/kg oral dose), it did not exhibit antiinflammatory activity. It is possible

that **14s** which has a SEt substitutent at the C-6 position of the pyranone ring may be more susceptible to metabolic inactivation compared to C-6 alkyl or alkoxy analogs when administered by the oral route which may result in a lower amount of the drug localization at the inflammation site. Studies using systemic routes of administration may provide more precise explanations for these observations which are not clear.

It is interesting that the C-6 alkyl (**14a, 14d** and **14e**), and the C-6 alkoxy (**14f, 14g** and **14i**) compounds increased rat paw edema volume when administered at a higher 5 and 10 mg/kg oral dose although the data obtained are not statistically significant. Over the years numerous studies employing rat acute models of inflammation have not been able to completely assess the contribution of the COX-2 enzyme in the inflammatory process. Recent studies have shown that in a carrageenan rat paw edema model, rofecoxib increased the levels of proinflammatory cytokine TNF-α in rat paw.[30] In addition, studies have implicated increased levels of inducible nitric oxide synthase in the carrageenan-induced rat paw inflammation assay which was not altered by the administration of COX-2 inhibitors.[31,32] Another recent article has shown the opposite effects of rofecoxib on activating protein (AP-1) and nuclear factor-κB (NF-κB) which may explain the lack of clear dose dependency, influencing both its wanted and unwanted effects.[33] Although the exact mechanisms for these observations are not clear, there are multiple factors that may be responsible for the increase in rat paw volume observed at higher doses for the compounds described in this study.

Analgesic activity was determined using the 4% NaCl-induced abdominal constriction assay. Compounds from the C-6 alkyl (**14a**, **14d** and **14e**) and C-6 alkoxy (**14f, 14g, 14i** and **14l**) subgroups exhibited a 20–83% inhibition of writhing at different time intervals with **14i** [6-ethoxy-3-(4-methanesulfonyl phenyl)-4-phenylpyran-2-one] exhibiting 42 and 75% inhibition in writhing at 30 and 60 min, respectively post-drug administration for a 1 mg/kg intraperitoneal dose (Table 3.1). 6-Ethylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (**14s**) was the most active analgesic agent where writhing was reduced by 81 and 77% inhibition at 30 and 60 min post-drug administration for a 50 mg/kg intraperitoneal dose relative to the reference drug celecoxib (31 and 62% inhibition at 30 and 60 min post-drug administration for a 50 mg/kg intraperitoneal dose). The good analgesic activity exhibited by C-6 alkyl (**14a**, **14d** and **14e**) and alkoxy (**14f, 14g** and **14i**) compounds at the 1 mg/kg intraperitoneal dose suggests roles for both peripheral and central actions of COX-2 inhibitors in alleviating pain in a wide variety of conditions.[34,35]

4.4.0.0. Conclusions

The qualitative structure–activity relationships acquired for this novel class of 6-alkyl (alkoxy or alkylthio)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones have shown that (i) a 6-membered lactone (pyran-2-one) ring serves as a suitable central ring template to design selective COX-2 inhibitors when the the C=O oxygen atom is suitably positioned to undergo hydrogen bonding to Tyr^{355} in the COX-2 binding site, (ii) the combined steric and electronic properties of the C-6 central substituted ring modulates COX-2

inhibitory potency and selectivity by orienting the C-3 *para*-SO_2Me phenyl substituent to the vicinity of the secondary pocket in the COX-2 binding site, vis a vis **14i** (C-6 ethoxy) and **14s** (C-6 ethylthio) exhibit excellent COX-2 inhibitory potency and selectivity, and (iii) the moderate to weak COX-1 inhibition exhibited by this class of compounds can be attributed partly to the steric hindrance of Ile^{523} at the entrance to the side pocket which blocks the access of the *para*-SO_2Me substituent, and (iv) the presence of a *para*-fluoro substituent on the C-4 phenyl ring improves in vivo antiinflammatory activity for this class of compounds.

4.5.0.0. Experimental Section

General. Melting points were determined using a Buchi capillary apparatus and are uncorrected. Ibuprofen was purchased from Sigma (St. Louis, MO). All other reagents including **10a–g** were purchased from Aldrich (Milwaukee, WI). Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70-230 mesh). Infrared (IR) spectra were recorded using a Nicolet 550 Series II Magna FT-IR spectrometer. Nuclear magnetic resonance (^1H NMR, ^{13}C NMR) spectra were recorded on a Bruker AM-300 spectrometer and chemical shifts are expressed in parts per million (ppm, δ) relative to tetramethylsilane as internal standard. Spin multiplets are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) and m (multiplet). Coupling constants (*J*) are given in Hertz (Hz). ^{13}C NMR spectra were acquired using the *J* modulated spin echo technique where methyl and methane carbons appear as positive peaks and methylene and quaternary carbon resonances appear as negative peaks. High Resolution Mass spectra (HRMS) were acquired using a Kratos MS-50 electron impact (EI) mass spectrometer. Microanalyses were performed for C, H and N (MicroAnalytical Service Laboratory, Department of Chemistry, University of Alberta) and were within ± 0.4% of the theoretical values. Celecoxib and rofecoxib were synthesized according to the literature procedures[5,14] Compounds **9a** [2-(4-methanesulfonylphenyl)-3-phenylcycloprop-2-en-1-one], and **9b** [2-(4-methanesulfonylphenyl)-3-(4-fluorophenyl)cycloprop-2-en-1-one], were prepared according to the previously reported method.[19] Male Spargue-Dawley rats, used in the antinflammatory-analgesic screens, were supplied by Animal Health Services, University of Alberta and experiments were carried out using protocols approved by the Animal Welfare Committee, University of Alberta.

General Procedure for the Synthesis of Alkyl haloacetates (10h–i) and alkyl halothioacetates (10j–m). To a stirred solution of either bromoacetyl bromide or chloroacetyl chloride (13.3 mmol) in dry diethyl ether (65 mL) was added 13.3 mmol of the respective alcohol (*i*-BuOH or 4-chlorobenzyl alcohol) or thiol (MeSNa, EtSH, *n*-PrSH or *i*-PrSH) at –23 °C (dry ice/acetone bath) under an argon atmosphere, and the reaction was allowed to proceed at 25 °C for 4 h. The reaction mixture was washed with 10% $NaHCO_3$ solution (30 mL), the organic phase was separated, and dried (Na_2SO_4). The solvent was removed either in vacuo, or under a stream of argon, to afford the respective alkyl haloacetate (**10h–i**), or

alkyl halothioacetate (**10j–m**) as a pale yellow oil in 59-85% yield. Products **10** were used immediately without further purification for the preparation of compounds **11**.

General Procedure for the Synthesis of *N*-Alkyl, alkoxy, or alkylthiocarbonylmethylpyridinium Halides (11a–m). To a stirred solution of dry pyridine (0.45 mL, 5.5 mmol) in dry THF (30 mL) under an argon atmosphere was added either an alkyl ketone (**10a–c**), alkyl haloacetate (**10d–i**), or alkyl halothioacetate (**10j–m**) (5.5 mmol), and the reaction mixture was stirred for 6 h at 25 °C. The solvent was either removed in vacuo, or under a stream of argon gas, and the solid or semisolid product obtained was purified by recrystallization from ethanol–acetone (1:1, v/v) to give the respective product (**11a–m**) in 33-88% yield. Some physical and spectroscopic (IR, ^{1}H NMR) data for **11a–m** are listed below.

***N*-(Methylcarbonylmethyl) pyridinium Chloride (11a).** This product was obtained as a white solid (0.41 g, 44%) by reaction of pyridine with **10a**: mp 205–206 °C (lit. 202–204 °C[36]); IR (KBr): 1658 cm^{-1} (C=O); ^{1}H NMR (DMSO–d_6): δ 2.32 (s, 3H, C*H*$_3$), 5.79 (s, 2H, N$^+$C*H*$_2$), 8.23 (dd, *J* = 7.9, *J* = 5.5 Hz, 2H, pyridine H-3, H-5), 8.68 (dd, *J* = 7.9, *J* = 7.9 Hz, 1H, pyridine H-4), 9.01 (d, *J* = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Ethylcarbonylmethyl) pyridinium Bromide (11b).** This product was obtained as a white solid (0.64 g, 50.7%) by reaction of pyridine with **10b**: mp 179–181 °C; IR (KBr): 1660 cm^{-1} (C=O); ^{1}H NMR (DMSO–d_6): δ 1.01 (t, *J* = 6.7 Hz, 3H, CH$_2$C*H*$_3$), 2.64 (q, *J* = 6.7 Hz, 2H, C*H*$_2$CH$_3$), 5.76 (s, 2H, N$^+$C*H*$_2$), 8.18 (dd, *J* = 7.9, *J* = 5.5 Hz, 2H, pyridine H-3, H-5), 8.64 (dd, *J* = 7.9, *J* = 7.9 Hz, 1H, pyridine H-4), 8.84 (d, *J* = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Isopropylcarbonylmethyl) pyridinium Bromide (11c).** This product was obtained as a solid (0.69 g, 51.4%) by reaction of pyridine with **10c**: mp 148–150 °C; IR (KBr) 1658 cm^{-1} (C=O); ^{1}H NMR (DMSO–d_6): δ 1.38 [d, *J* = 6.7 Hz, 6H, CH(C*H*$_3$)$_2$], 3.76-3.83 [m, 1H, C*H*(CH$_3$)$_2$], 6.00 (s, 2H, N$^+$C*H*$_2$), 8.27 (dd, *J* = 7.9, *J* = 5.5 Hz, 2H, pyridine H-3, H-5), 8.74 (dd, *J* = 7.9, *J* = 7.9 Hz, 1H, pyridine H-4), 9.08 (d, *J* = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Methoxycarbonylmethyl) pyridinium Bromide (11d).** This product was obtained as a white solid (0.41 g, 32.4%) by reaction of pyridine with **10d**: mp 176–178 °C (lit. 174–175°C[37]); IR (KBr): 1745 cm^{-1} (C=O); ^{1}H NMR (DMSO–d_6): δ 3.75 (s, 3H, OC*H*$_3$), 5.93 (s, 2H, N$^+$C*H*$_2$), 8.04 (dd, *J* = 7.9, *J* = 5.5 Hz, 2H, pyridine H-3, H-5), 8.54 (dd, *J* = 7.9, *J* = 7.9 Hz, 1H, pyridine H-4), 9.30 (d, *J* = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Ethoxycarbonylmethyl) pyridinium Bromide (11e).** This product was obtained as a white solid (0.43 g, 31.8%) by reaction of pyridine with **10e**: mp 132–134 °C (lit. 134–136 °C[37]); IR (KBr): 1750 cm^{-1} (C=O); ^{1}H NMR (DMSO–d_6): δ 1.23 (t, *J* = 6.7 Hz, 3H, OCH$_2$C*H*$_3$), 4.20 (q, *J* = 6.7 Hz, 2H, OC*H*$_2$CH$_3$), 5.65 (s, 2H, N$^+$C*H*$_2$), 8.22 (dd, *J* = 7.9, *J* = 5.5 Hz, 2H, pyridine H-3, H-5), 8.69 (dd, *J* = 7.9, *J* = 7.9 Hz, 1H,

pyridine H-4), 9.04 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Isopropyloxycarbonylmethyl) pyridinium Bromide (11f).** This product was obtained as a white solid (0.92 g, 65%) by reaction of pyridine with **10f**: mp 90–91 °C (lit. 79–81°C[37]); IR (KBr) 1751 cm^{-1} (C=O); ^1H NMR (DMSO–d_6): δ 1.25 [d, J = 6.7 Hz, 6H, $OCH(CH_3)_2$], 5.01-5.13 [m, 1H, $OCH(CH_3)_2$], 5.72 (s, 2H, N^+CH_2), 8.27 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.74 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.14 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(*tert*-Butoxycarbonylmethyl) pyridinium Bromide (11g).** This product was obtained as a semi–solid (0.70 g, 47%) by reaction of pyridine with **10g**: IR (KBr): 1746 cm^{-1} (C=O); ^1H NMR (DMSO–d_6): δ 1.58 [s, 9H, $OC(CH_3)_3$], 5.83 (s, 2H, N^+CH_2), 8.27 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.73 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.14 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Isobutoxycarbonylmethyl) pyridinium Bromide (11h).** This product was obtained as a white solid (1.0 g, 68%) by reaction of pyridine with **10h**: mp 86–88 °C; IR (KBr): 1748 cm^{-1} (C=O); ^1H NMR (DMSO–d_6): δ 0.88 [d, J = 6.7 Hz, 6H, $OCH_2CH(CH_3)_2$], 1.84–1.95 [m, 1H, $OCH_2CH(CH_3)_2$], 3.96 [d, J = 6.7 Hz, 2H, $OCH_2CH(CH_3)_2$], 5.80 (s, 2H, N^+CH_2), 8.27 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.74 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.15 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(4-Chlorobenzyloxycarbonyl methyl) pyridinium Bromide (11i).** This product was obtained as a semi–solid (0.71 g, 38%) by reaction of pyridine with **10i**: IR (KBr): 1746 cm^{-1} (C=O); ^1H NMR (DMSO–d_6): δ 5.26 (s, 2H, 4-Cl-C_6H_4-CH_2), 5.79 (s, 2H, N^+CH_2), 7.41 (d, J = 8.5 Hz, 2H, 4-chlorophenyl H-2, H-6), 7.45 (d, J = 8.5 Hz, 2H, 4-chlorophenyl H-3, H-5), 8.26 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.73 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.14 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Methylthiocarbonylmethyl) pyridinium Bromide (11j).** This product was obtained as a white solid (0.93 g, 68.3%) by reaction of pyridine with **10j**: mp 225–226 °C; IR (KBr): 1667 cm^{-1} (C=O); ^1H NMR (DMSO–d_6): δ 2.5 (s, 3H, SCH_3), 5.99 (s, 2H, N^+CH_2), 8.23 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.66 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.02 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6). Anal. ($C_8H_{10}BrNOS$): C, H, N.

***N*-(Ethylthiocarbonylmethyl) pyridinium Bromide (11k).** This product was obtained as a white solid (0.50 g, 33%) by reaction of pyridine with **10k**: mp 173–175 °C; IR (KBr): 1673 cm^{-1} (C=O); ^1H NMR (DMSO–d_6): δ 1.24 (t, J = 6.7 Hz, 3H, SCH_2CH_3), 3.00 (q, J = 6.7 Hz, 2H, SCH_2CH_3), 5.99 (s, 2H, N^+CH_2), 8.26 (dd, J = 7.9, J = 5. 5 Hz, 2H, pyridine H-3, H-5), 8.73 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.05 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Propylthiocarbonylmethyl) pyridinium Bromide (11l).** This product was obtained as a viscous oil (0.65 g, 43%) by reaction of pyridine with **10l**: IR (KBr): 1675 cm^{-1} (C=O); ^1H, 3H, $SCH_2CH_2CH_3$), 1.52-1.55 (m, 2H,

$SCH_2CH_2CH_3$), 2.96 (t, 2H, J = 7.0 Hz, $SCH_2CH_2CH_3$), 6.00 (s, 2H, N^+CH_2), 8.23 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.70 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.04 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

***N*-(Isopropylthiocarbonylmethyl) pyridinium Bromide (11m).** This product was obtained as a semi-solid (0.57 g, 38%) by reaction of pyridine with **10m**: IR (KBr): 1672 cm^{-1} (C=O); ^1H NMR (DMSO-d_6): δ 1.38 [d, J = 6.7 Hz, 6H, $SCH(CH_3)_2$], 3.76-3.83 [m, 1H, $SCH(CH_3)_2$], 6.00 (s, 2H, N^+CH_2), 8.27 (dd, J = 7.9, J = 5.5 Hz, 2H, pyridine H-3, H-5), 8.74 (dd, J = 7.9, J = 7.9 Hz, 1H, pyridine H-4), 9.08 (d, J = 5.5 Hz, 2H, pyridine H-2, H-6).

General Procedure for the Synthesis of 6-Alkyl, alkoxy, or alkylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (14a–v). To a solution of the *N*-alkyl, alkoxy, or alkylthiocarbonylmethylpyridinium salt selected (**11a–m**, 2.4 mmol) in dry benzene (60 mL) at 25 °C was added freshly distilled triethylamine (1 mL, 7 mmol). To this reaction mixture, 2-(4-methanesulfonylphenyl)-3-phenylcycloprop-2-en-1-one (**9a**, 0.29 g, 1 mmol), or 2-(4-methanesulfonylphenyl)-3-(4-fluorophenyl)cycloprop-2-en-1-one (**9b**, 0.31 g, 1 mmol), was added and the mixture was stirred for 16-18 h at 25 °C. The solvent was evaporated under reduced pressure and the residue obtained was purified by silica gel column chromatography using hexane–ethyl acetate (1:2, v/v or 1:3, v/v) as eluent to afford the respective title compound **14a–v** in 8–45% yield. Some physical and spectroscopic data for **14a–v** are listed below.

6-Methyl-3-(4-methanesulfonyl phenyl)-4-phenylpyran-2-one (14a). The product was obtained as a solid by condensation of **9a** with **11a** in the presence of triethylamine (0.03 g, 9%): mp 140–141 °C; IR (KBr): 1710 (C=O), 1306, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.38 (s, 3H, CH_3), 3.02 (s, 3H, SO_2CH_3), 6.24 (s, 1H, pyranone H-5), 7.04–7.07 (m, 2H, phenyl H-2, H-6), 7.22–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.36 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{19}H_{16}O_4S$): C, H.

4-(4-Fluorophenyl)-6-methyl-3-(4-methanesulfonylphenyl)pyran-2-one (14b). The product was obtained as a solid by condensation of **9b** with **11a** in the presence of triethylamine (0.03 g, 8%): mp 156–158 °C; IR (KBr): 1712 (C=O), 1313, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.38 (s, 3H, CH_3), 3.03 (s, 3H, SO_2CH_3), 6.20 (s, 1H, pyranone H-5), 6.94 (dd, J^3_{HH} = 8.4, J^3_{FH} = 8.4 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.05 (dd, J^3_{HH} = 8.4, J^3_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.32 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.70 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{19}H_{15}FO_4S$): C, H.

6-Ethyl-3-(4-methanesulfonyl phenyl)-4-phenylpyran-2-one (14c). The product was obtained as a solid by condensation of **9a** with **11b** in the presence of triethylamine (0.14 g, 38.3%): mp 172–174 °C; IR (KBr): 1709

(C=O), 1313, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.30 (t, J = 7.3 Hz, 3H, CH_2CH_3), 2.62 (q, J = 7.3 Hz, 2H, CH_2CH_3), 3.02 (s, 3H, SO_2CH_3), 6.22 (s, 1H, pyranone H-5), 7.04–7.08 (m, 2H, phenyl H-2, H-6), 7.22–7.33 (m, 3H, phenyl H-3, H-4, H-5), 7.35 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.77 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{20}H_{18}O_4S$): C, H.

6-Isopropyl-3-(4-methanesulfonyl phenyl)-4-phenylpyran-2-one (14d). The product was obtained by condensation of **9a** with **11c** in the presence of triethylamine (0.05 g, 14%): mp 174–175 °C; IR (KBr): 1703 (C=O), 1307, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.34 [d, J = 7.0 Hz, 6H, $CH(CH_3)_2$], 2.86–2.91 [m, 1H, $CH(CH_3)_2$], 3.03 (s, 3H, SO_2CH_3), 6.21 (s, 1H, pyranone H-5), 7.05–7.08 (m, 2H, phenyl H-2, H-6), 7.23–7.34 (m, 3H, phenyl H-3, H-4, H-5), 7.36 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{21}H_{20}O_4S$): C, H.

4-(4-Fluorophenyl)-6-isopropyl-3-(4-methanesulfonylphenyl)pyran-2-one (14e). The product was obtained by condensation of **9b** with **11c** in the presence of triethylamine (0.06 g, 16%): mp 188–189 °C; IR (KBr): 1710 (C=O), 1312, 1148 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.36 [d, J = 7.0 Hz, 6H, $CH(CH_3)_2$], 2.87–2.93 [m, 1H, $CH(CH_3)_2$], 3.05 (s, 3H, SO_2CH_3), 6.13 (s, 1H, pyranone H-5), 6.98 (dd, J^3_{HH} = 8.7, J^3_{FH} = 8.7 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.07 (dd, J^3_{HH} = 8.7, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.38 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.83 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5. Anal. ($C_{21}H_{19}FO_4S$): C, H.

6-Methoxy-3-(4-methanesulfonyl phenyl)-4-phenylpyran-2-one (14f). The product was obtained as a solid by condensation of **9a** with **11d** in the presence of triethylamine (0.04 g, 12%): mp 110–112 °C; IR (KBr): 1719 (C=O), 1310, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.02 (s, 3H, SO_2CH_3), 4.03 (s, 3H, OCH_3), 5.59 (s, 1H, pyranone H-5), 7.06–7.09 (m, 2H, phenyl H-2, H-6), 7.21–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.33 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{19}H_{16}O_5S$): C, H.

4-(4-Fluorophenyl)-6-methoxy-3-(4-methanesulfonylphenyl)pyran-2-one (14g). The product was obtained as a solid by condensation of **9b** with **11d** in the presence of triethylamine (0.05 g, 14%): mp 148–149 °C; IR (KBr): 1720 (C=O), 1315, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.03 (s, 3H, SO_2CH_3), 4.04 (s, 3H, OCH_3), 5.56 (s, 1H, pyranone H-5), 6.93 (dd, J^3_{HH} = 8.4, J^3_{FH} = 8.4 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.07 (dd, J^3_{HH} = 8.4, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.33 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{19}H_{15}FO_5S$): C, H.

6-Ethoxy-3-(4-methanesulfonyl-phenyl)-4-phenylpyran-2-one (14h). The product was obtained as a solid by condensation of **9a** with **11e** in the presence of triethylamine (0.06 g, 16%): mp 174–176 °C; IR (KBr): 1719 (C=O),

1310, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.46 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 3.02 (s, 3H, SO_2CH_3), 4.32 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 5.57 (s, 1H, pyranone H-5), 7.07–7.08 (m, 2H, phenyl H-2, H-6), 7.18–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.35 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.74 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 14.7 (OCH_2CH_3), 44.5 (SO_2CH_3), 65.7 (OCH_2CH_3), 107.2 (pyranone C-5), 112.7 (pyranone C-3), 126.8 (phenyl C-3, C-5), 128.4 and 128.7 (phenyl C-2, C-6; 4-methanesulfonylphenyl C-2, C-6), 129.2 (phenyl C-4), 132.1 (4-methanesulfonylphenyl C-3, C-5), 137.4 (phenyl C-1), 138.7 (4-methanesulfonylphenyl C-1), 140.4 (4-methanesulfonylphenyl C-4), 159.0 (pyranone C-4), 160.6 (pyranone C-6), 162.7 (pyranone C-2); HRMS m/z calcd for $C_{20}H_{18}O_5S$, 370.08749, found 370.08729. Anal. ($C_{20}H_{18}O_5S$): C, H.

4-(4-Fluorophenyl)-6-ethoxy-3-(4-methanesulfonylphenyl)pyran-2-one (14i). The product was obtained as a solid by condensation of **9b** with **11e** in the presence of triethylamine (0.08 g, 20%): mp 154–156 °C; IR (KBr): 1719 (C=O), 1312, 1146 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.49 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 3.03 (s, 3H, SO_2CH_3), 4.38 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 5.55 (s, 1H, pyranone H-5), 6.95 (dd, J^3_{HH} = 8.3, J^3_{FH} = 8.3 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.08 (dd, J^3_{HH} = 8.3, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.33 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{20}H_{17}FO_5S$): C, H.

6-Isopropyloxy-3-(4-methane sulfonylphenyl)-4-phenylpyran-2-one (14j). The product was obtained as a solid by condensation of **9a** with **11f** in the presence of triethylamine (0.10 g, 24%): mp 185–187 °C; IR (KBr): 1720 (C=O), 1319, 1140 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.46 [d, J = 7.0 Hz, 6H, $OCH(CH_3)_2$], 3.02 (s, 3H, SO_2CH_3), 5.06–5.13 [m, 1H, $OCH(CH_3)_2$], 5.58 (s, 1H, pyranone H-5), 7.04–7.08 (m, 2H, phenyl H-2, H-6), 7.20–7.32 (m, 3H, phenyl H-3, H-4, H-5), 7.32 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{21}H_{20}O_5S$): C, H.

4-(4-Fluorophenyl)-6-isopropyloxy-3-(4-methanesulfonylphenyl)pyran-2-one (14k). The product was obtained as a solid by condensation of **9b** with **11f** in the presence of triethylamine (0.09 g, 21%): mp 180–181 °C; IR (KBr): 1722 (C=O), 1310, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.46 [d, J = 6.1 Hz, 6H, $OCH(CH_3)_2$], 3.03 (s, 3H, SO_2CH_3), 5.06–5.13 [m, 1H, $OCH(CH_3)_2$], 5.55 (s, 1H, pyranone H-5), 6.94 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.06 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.33 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{21}H_{19}FO_5S$): C, H.

6-*tert*-Butoxy-3-(4-methanesulfonyl phenyl)-4-phenylpyran-2-one (14l). The product was obtained as solid by condensation of **9a** with **11g** in the presence of triethylamine (0.10 g, 25%): mp 129–131 °C; IR (KBr): 1719 (C=O), 1310, 1146 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.63 [s, 9H, $OC(CH_3)_3$], 3.02

(s, 3H, SO_2CH_3), 5.68 (s, 1H, pyranone H-5), 7.04–7.07 (m, 2H, phenyl H-2, H-6), 7.21–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.38 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{22}H_{22}O_5S$): C, H.

6-*tert*-Butoxy-4-(4-fluorophenyl)-3-(4-methanesulfonylphenyl)pyran-2-one (14m). The product was obtained as a solid by condensation of **9b** with **11g** in the presence of triethylamine (0.09 g, 18%): mp 132–133 °C; IR (KBr): 1722 (C=O), 1310, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.55 [s, 9H, $OC(CH_3)_3$], 3.01 (s, 3H, SO_2CH_3), 5.56 (s, 1H, pyranone H-5), 6.95 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.07 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.25 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.70 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{22}H_{21}FO_5S$): C, H.

6-Isobutoxy-3-(4-methanesulfonyl phenyl)-4-phenylpyran-2-one (14n). The product was obtained as a solid by condensation of **9a** with **11h** in the presence of triethylamine (0.09 g, 22%): mp 156–158 °C; IR (KBr): 1720 (C=O), 1311, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.05 [d, J = 6.7 Hz, 6H, $OCH_2CH(CH_3)_2$], 2.11–2.19 [m, 1H, $OCH_2CH(CH_3)_2$], 3.01 (s, 3H, SO_2CH_3), 4.03 [d, J = 6.7 Hz, 2H, $OCH_2CH(CH_3)_2$], 5.58 (s, 1H, pyranone H-5), 7.05–7.09 (m, 2H, phenyl H-2, H-6), 7.20–7.30 (m, 3H, phenyl H-3, H-4, H-5), 7.34 (d, J = 8.0 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.0 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{22}H_{22}O_5S$): C, H.

4-(4-Fluorophenyl)-6-isobutoxy-3-(4-methanesulfonylphenyl)pyran-2-one (14o). The product was obtained as a solid by condensation of **9b** with **11h** in the presence of triethylamine (0.10 g, 24%): mp 138–139 °C; IR (KBr): 1719 (C=O), 1310, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.06 [d, J = 6.7 Hz, 6H, $OCH_2CH(CH_3)_2$], 2.12–2.18 [m, 1H, $OCH_2CH(CH_3)_2$], 3.03 (s, 3H, SO_2CH_3), 4.06 [d, J = 6.7 Hz, 2H, $OCH_2CH(CH_3)_2$], 5.55 (s, 1H, pyranone H-5), 6.95 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.07 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.32 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.79 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{22}H_{21}FO_5S$): C, H.

6-(4-Chlorobenzyloxy)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14p). The product was obtained as a solid by condensation of **9a** with **11i** in the presence of triethylamine (0.08 g, 16%): mp 157–159 °C; IR (KBr): 1724 (C=O), 1310, 1146 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.02 (s, 3H, SO_2CH_3), 5.32 (s, 2H, 4-Cl-C_6H_4-CH_2O), 5.66 (s, 1H, pyranone H-5), 7.03 (m, 2H, phenyl H-2, H-6), 7.13–7.24 (m, 3H, phenyl H-3, H-4, H-5), 7.27 (d, 2H, J = 8.4 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.31 (d, J = 8.5 Hz, 2H, 4-chlorophenyl H-2, H-6), 7.37 (d, J = 8.5 Hz, 2H, 4-chlorophenyl H-3, H-5), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{25}H_{19}ClO_5S$): C, H.

6-(4-Chlorobenzyloxy)-4-(4-fluorophenyl)-3-(4-methanesulfonylphenyl)pyran-2-one (14q). The product was obtained as a solid by condensation of **9b** with **11i** in the presence of triethylamine (0.07 g, 14%): mp 173–174 °C; IR (KBr): 1722 (C=O), 1310, 1148 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.03 (s, 3H, SO_2CH_3), 5.32 (s, 2H, 4-Cl-C_6H_4-CH_2O), 5.63 (s, 1H, pyranone H-5), 6.94 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.05 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.32 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.41 (d, J = 8.5 Hz, 2H, 4-chlorophenyl H-2, H-6), 7.44 (d, J = 8.5 Hz, 2H, 4-chlorophenyl, H-3, H-5), 7.79 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{25}H_{18}ClFO_5S$): C, H.

6-Methylthio-3-(4-methanesulfonyl phenyl)-4-phenylpyran-2-one (14r). The product was obtained as a solid by condensation of **9a** with **11j** in the presence of triethylamine (0.14 g, 34.3%): mp 207–208 °C; IR (KBr): 1725 (C=O), 1308, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.6 (s, 3H, SCH_3), 3.02 (s, 3H, SO_2CH_3), 6.29 (s, 1H, pyranone H-5), 7.05–7.07 (m, 2H, phenyl H-2, H-6), 7.22–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.33 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.76 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{19}H_{16}O_4S_2.1/2H_2O$): C, H.

6-Ethylthio-3-(4-methanesulfonyl phenyl)-4-phenylpyran-2-one (14s). The product was obtained as a solid by condensation of **9a** with **11k** in the presence of triethylamine (0.06 g, 15%): mp 175–176 °C; IR (KBr): 1718 (C=O), 1314, 1153 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.46 (t, J = 7.3 Hz, 3H, SCH_2CH_3), 3.03 (s, 3H, SO_2CH_3), 3.15 (q, J = 7.3 Hz, 2H, SCH_2CH_3), 6.35 (s, 1H, pyranone H-5), 7.04–7.07 (m, 2H, phenyl H-2, H-6), 7.21–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.35 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 14.7 (SCH_2CH_3), 25.8 (SCH_2CH_3), 44.5 (SO_2CH_3), 107.3 (pyranone C-5), 117.6 (pyranone C-3), 126.9 (phenyl C-3, C-5), 128.4 and 128.6 (phenyl C-2, C-6; 4-methanesulfonylphenyl C-2, C-6), 129.3 (phenyl C-4), 131.9 (4-methanesulfonylphenyl C-3, C-5), 136.5 (phenyl C-1), 139.2 (4-methanesulfonylphenyl C-1), 139.9 (4-methanesulfonylphenyl C-4), 154.3 (pyranone C-4), 161.7 (pyranone C-6), 162.2 (pyranone C-2). Anal. ($C_{20}H_{18}O_4S_2$): C, H.

4-(4-Fluorophenyl)-6-ethylthio-3-(4-methanesulfonylphenyl)pyran-2-one (14t). The product was obtained as a solid by condensation of **9b** with **11k** in the presence of triethylamine (0.07 g, 17%): mp 171–172 °C; IR (KBr): 1716 (C=O), 1313, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.46 (t, J = 7.3 Hz, 3H, SCH_2CH_3), 3.04 (s, 3H, SO_2CH_3), 3.15 (q, J = 7.3 Hz, 2H, SCH_2CH_3), 6.32 (s, 1H, pyranone H-5), 6.96 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.07 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.35 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.80 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{20}H_{17}FO_4S_2$): C, H.

3-(4-Methanesulfonylphenyl)-6-*n*-propylthio-4-phenylpyran-2-one (14u).

The product was obtained as a solid by condensation of **9a** with **11l** in the presence of triethylamine (0.14 g, 32%): mp 158–160 °C; IR (KBr): 1727 (C=O), 1308, 1157 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.06 (t, J = 7.3 Hz, 3H, $SCH_2CH_2CH_3$), 1.56–1.87 (m, 2H, $SCH_2CH_2CH_3$), 3.02 (s, 3H, SO_2CH_3), 3.09 (t, J = 7.3 Hz, 2H, $SCH_2CH_2CH_3$), 6.35 (s, 1H, pyranone H-5), 7.04–7.06 (m, 2H, phenyl H-2, H-6), 7.22–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.34 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.76 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 13.3 ($SCH_2CH_2CH_3$), 22.9 ($SCH_2CH_2CH_3$), 33.2 ($SCH_2CH_2CH_3$), 44.5 (SO_2CH_3), 107.2 (pyranone C-5), 117.4 (pyranone C-3), 126.9 (phenyl C-3, C-5), 128.4 and 128.8 ((phenyl C-2, C-6; 4-methanesulfonylphenyl C-2, C-6), 129.3 (phenyl C-4), 131.9 (4-methanesulfonylphenyl C-3, C-5), 136.4 (phenyl C-1), 139.0 (4-methanesulfonylphenyl C-1), 139.9 (4-methanesulfonylphenyl C-4), 154.3 (pyranone C-4), 161.9 (pyranone C-6), 162.3 (pyranone C-2). Anal ($C_{21}H_{20}O_4S_2$): C, H.

6-Isopropylthio-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (14v). The product was obtained by condensation of **9a** with **11m** in the presence of triethylamine (0.19 g, 44.5%): mp 164–166 °C; IR (KBr): 1721 (C=O), 1310, 1160 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.42 [d, J = 6.7 Hz, 6H, $SCH(CH_3)_2$], 3.01 (s, 3H, SO_2CH_3), 3.83–3.92 [m, 1H, $SCH(CH_3)_2$], 6.39 (s, 1H, pyranone H-5), 7.02–7.05 (m, 2H, phenyl H-2, H-6), 7.20–7.29 (m, 3H, phenyl H-3, H-4, H-5), 7.33 (d, J = 8.5 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{21}H_{20}O_4S_2$): C, H.

Cyclooxygenase Inhibition Studies. All compounds described herein were tested for their ability to inhibit COX-1 and COX-2 using a COX-(ovine) inhibitor screening kit (Catalog No. 560101, Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions. Cyclooxygenase catalyzes the first step in the biosynthesis of arachidonic acid (AA) to PGH_2. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, is measured by enzyme immunoassay (ACE™ competitive EIA). Stock solutions of test compounds were dissolved in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (960 µl, 0.1 M Tris-HCl pH 8.0 containing 5 mM EDTA and 2 mM phenol) with either COX-1 or COX-2 (10 µl) enzyme in presence of heme (10 µl) were added 10 µl of various concentrations of test drug solutions (0.001, 0.01, 0.1, 1, 10, 100, and 500 µM in a final volume of 1 mL). These solution were incubated for a period of 2 min at 37 °C after which 10 µl of AA (100 µM) was added and the COX reaction was stopped by the addition of 50 µl of 1M HCl after 2 min. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, was measured by enzyme immunoassay. This assay is based on the competition between PGs and a PG-acetylcholinesterase conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells since the concentration of the PG tracer is held

constant while the concentration of PGs varies. This antibody-PG complex binds to a mouse anti-rabbit monoclonal antibody that had been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's Reagent, which contains the substrate to acetylcholinesterase, is added to the well. The product of this enzymatic reaction produces a distinct yellow color that absorbs at 405 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of PGs present in the well during the incubation: Absorbance $\propto$ [Bound PG Tracer] $\propto$ 1/PGs. Percent inhibition was calculated by comparison of compound-treated to various control incubations. The concentration of the test compound causing 50% inhibition (IC_{50}, μM) was calculated from the concentration-inhibition response curve (duplicate determinations).

Antiinflammatory Assay. The test compounds were evaluated using the in vivo rat carrageenan-induced foot paw edema model reported previously.[38,39]

Analgesic Assay. Analgesic activity was determined using the 4% sodium chloride-induced writhing (abdominal constriction) assay as described previously.[40,41]

Molecular Modeling (Docking) Studies. Docking experiments were performed using Insight II software Version 2000.1 (Accelrys Inc.) running on a Silicon Graphics Octane 2 R14000A workstation. The coordinates for the X-ray crystal structures of the enzymes COX-1 and COX-2 were obtained from the RCSB Protein Data Bank and hydrogens were added. The ligand molecules were constructed using the Builder module and then energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The docking experiment on COX-2 was carried out by superimposing the energy minimized ligand on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. In the case of COX-1 (PDB file 1prh), the ligand was suitably positioned in the active site while carefully monitoring nonbonded interactions of the ligand–enzyme assembly and any side chain bumps. The resulting ligand–enzyme complex was subjected to docking using the Affinity command in the Docking module of Insight II after defining subsets of the enzyme such that residues within 10 Å of the ligand were allowed to relax, while the remainder of the enzyme residues were fixed. The consistent valence force field (CVFF) was employed for all docking purposes. The optimal binding orientation of the ligand–enzyme assembly obtained after docking was further minimized for 1000 iterations using the conjugate gradient method until a convergence of 0.001 kcal/mol Å was reached. The ligand–enzyme assembly was then subjected to a molecular dynamics (MD) simulation using the Discover module Version 2.98 at a constant temperature of 300 K with a 200 step equilibration for over 5000 iterations and a time step of 1 fs using a distance dependent dielectric constant 4r.

Supporting Information on Elemental Analysis Data is Available in Appendix 3.1.

4.6.0.0. References

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Appendix 3.2:

Elemental Analysis for Compounds **11j** and **14a–v**.

Cmpd	Empirical Formula	Calculated			Found		
		C	H	N	C	H	N
11j	$C_8H_{10}BrNOS$	38.68	4.02	5.64	38.54	3.94	5.51
14a	$C_{19}H_{16}O_4S$	67.04	4.73	–	67.28	4.96	–
14b	$C_{19}H_{15}FO_4S$	63.67	4.22	–	63.39	4.17	–
14c	$C_{20}H_{18}O_4S$	67.78	5.12	–	67.35	5.14	–
14d	$C_{21}H_{20}O_4S$	68.27	5.45	–	68.46	5.32	–
14e	$C_{21}H_{19}FO_4S$	65.27	4.95	–	65.34	4.82	–
14f	$C_{19}H_{16}O_5S$	64.03	4.52	–	63.97	4.66	–
14g	$C_{19}H_{15}FO_5S$	60.95	4.03	–	60.73	4.21	–
14h	$C_{20}H_{18}O_5S.1/3\ H_2O$	63.82	4.87	–	63.76	4.70	–
14i	$C_{20}H_{17}FO_5S$	61.84	4.41	–	61.78	4.32	–
14j	$C_{21}H_{20}O_5S$	65.61	5.24	–	65.46	5.21	–
14k	$C_{21}H_{19}FO_5S$	62.67	4.76	–	62.41	4.54	–
14l	$C_{22}H_{22}O_5S$	66.31	5.56	–	66.24	5.47	–
14m	$C_{22}H_{21}FO_5S$	63.44	5.08	–	63.47	5.33	–
14n	$C_{22}H_{22}O_5S$	66.31	5.56	–	66.28	5.31	–
14o	$C_{22}H_{21}FO_5S$	63.44	5.08	–	63.06	4.91	–
14p	$C_{25}H_{19}ClO_5S$	64.31	4.10	–	64.18	3.99	–
14q	$C_{25}H_{18}ClFO_5S$	61.92	3.74	–	61.87	3.46	–
14r	$C_{19}H_{16}O_4S_2 \cdot 1/2\ H_2O$	59.77	4.19	–	59.83	4.25	–

14s	$C_{20}H_{18}O_4S_2$	62.16	4.69	–	62.04	4.85	–
14t	$C_{20}H_{17}FO_4S_2$	59.39	4.20	–	59.11	4.17	–
14u	$C_{21}H_{20}O_4S_2$	62.98	5.03	–	62.63	4.99	–
14v	$C_{21}H_{20}O_4S_2$	62.98	5.03	–	62.76	5.06	–

5.0.0.0. CHAPTER 4.0

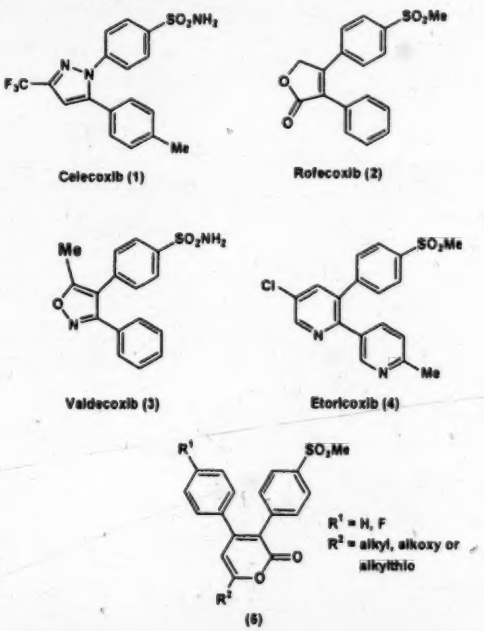
Design, Synthesis and Structure-Activity Relationship (SAR) Studies of 3,4,6-Triphenylpyran-2-ones as Selective Cyclooxygenase-2 Inhibitors

5.1.0.0. Introduction

The discovery of another isoform of the enzyme cyclooxygenase (COX-2) in the early 1990's led to the development of a new class of nonsteroidal antiinflammatory drugs (NSAIDs) known as selective cyclooxygenase-2 (COX-2) inhibitors.[1-3] Recent studies have shown that selective COX-2 inhibitors are equally effective in the treatment of inflammatory diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA) with an improved gastrointestinal (GI) profile compared to traditional NSAIDs.[4,5] In recent years there has been significant advancement in drug design concepts regarding selective COX-2 inhibitors and their potential application for the treatment of a variety of disease states. For example, the treatment of colon, breast and prostate cancer have shown promising results.[6,7] In addition, recent studies have highlighted the potential application of selective COX-2 inhibitors in the prophylactic prevention of neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease.[8,9]

Diarylheterocycles constitute a major class of selective COX-2 inhibitors. Extensive structure-activity relationship (SAR) studies for the diarylheterocycle class have shown that a SO_2NH_2 or SO_2Me substituent at the *para*-position of one of the aryl rings is a requirement for optimum COX-2 selectivity and potency.[10] Accordingly, the selective COX-2 inhibitor celecoxib (1), possesses a diarylheterocyclic ring template with a central 5-membered pyrazole ring, whereas rofecoxib (**2**), has a central 5-membered lactone [2-(5*H*)furanone], ring system.[11,12] Similarly, the recently launched selective COX-2 inhibitor valdecoxib (**3**), possesses a diarylheterocyclic ring template with a central 5-membered isoxazole ring, whereas etoricoxib (**4**), possesses a central 6-membered pyridine ring.[13-15]

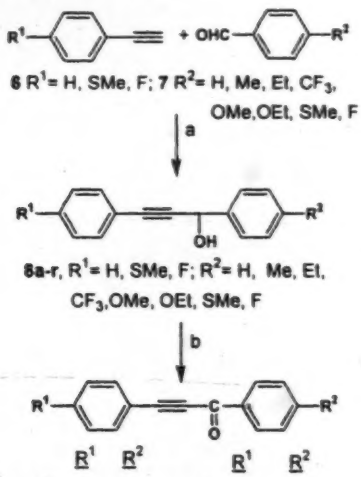
Chart 4.1: Representative examples of tricyclic selective COX-2 inhibitors.



Recent studies have shown that compounds possessing a pyran-2-one moiety are known to exhibit diverse biological activities including anticancer, antimicrobial and HIV-protease

inhibitory activities.[16-18] We have shown that a novel class of diarylheterocycle possessing a central pyran-2-one ring (**5**, see Chart 4.1), acts as a suitable template for the design of selective COX-2 inhibitors that exhibited good antiinflammatory and analgesic activity profiles.[19] In addition, other studies have also used a central pyran-2-one ring template for the design of selective COX-2 inhibitors.[20,21] As part of our ongoing research program, we describe herein the design, synthesis and structure-activity relationship (SAR) studies for a novel class of regioisomeric, 3,4,6-triphenylpyran-2-ones as selective COX-2 inhibitors.

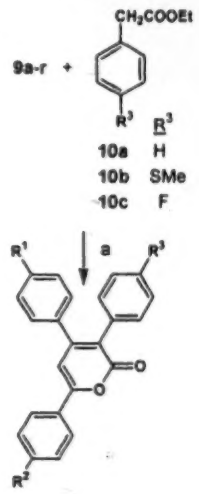
5.2.0.0. Chemistry



	R¹	R²		R¹	R²
9a	H	H	**9j**	F	OEt
9b	H	Me	**9k**	F	SMe
9c	H	Et	**9l**	SMe	H
9d	H	CF_3	**9m**	SMe	Me
9e	H	OMe	**9n**	SMe	Et
9f	H	OEt	**9o**	SMe	CF_3
9g	H	SMe	**9p**	SMe	OMe
9h	H	F	**9q**	SMe	OEt
9i	F	OMe	**9r**	SMe	F

Scheme 4.1: Reagents and conditions: (a) THF, -78 °C, *n*-BuLi, and then at -78 °C to 25 °C over night; (b) acetone, MnO_2, 25 °C, 4–5 h.

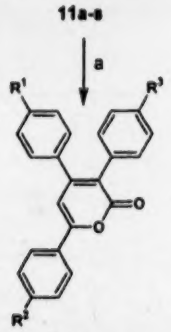
A number of methods for synthesizing 3,4,6-triphenylpyran-2-one derivatives have been reported.[22-24] The synthetic methods used to prepare the regioisomeric 3,4,6-triphenylpyran-2-ones (**12a–s**) are outlined in Schemes 4.1, 4.2 and 4.3. The initial strategy was to synthesize the 1,3-diarylprop-2-yn-1-ols (**8a–r**), which was achieved by the condensation of a *para*-substituted-phenylacetylene (**6**) with a *para*-substituted-benzaldehyde (**7**) in the presence of *n*-butyllithium (44–84%).[25] Subsequent oxidation of **8a–r** using activated manganese dioxide (MnO_2) afforded the corresponding 1,3-diarylprop-2-yn-1-one (**9a–r**) in good yield (52–85%) as shown in Scheme 4.1.



	R¹	R²	R³		R¹	R²	R³
11a	H	H	SMe	**11k**	SMe	H	H
11b	H	Me	SMe	**11l**	SMe	Me	H
11c	H	Et	SMe	**11m**	SMe	Et	H
11d	H	CF_3	SMe	**11n**	SMe	CF_3	H
11e	H	OMe	SMe	**11o**	SMe	OMe	H
11f	H	OEt	SMe	**11p**	SMe	OEt	H
11g	H	F	SMe	**11q**	SMe	F	H
11h	F	OMe	SMe	**11r**	SMe	OMe	F
11i	F	OEt	SMe	**11s**	SMe	OEt	F
11j	H	SMe	H				

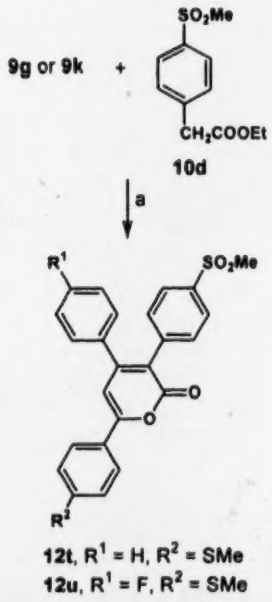
Scheme 4.2: Reagents and conditions: (a) DMSO, NaH, 25 °C, 1 h.

The 3,4,6-triphenylpyran-2-ones (**11a–s**) having a central 6-membered lactone ring were obtained in moderate to good yields (22–62%) by condensation of a *para*-substituted-phenylacetic acid ester (**10a–c**) with a 1,3-diarylprop-2-yn-1-one (**9a–j** or **9l–r**) in the presence of a base such as sodium hydride at 25 °C (Scheme 4.2).[22] Oxidation of **11a–s**, using an aqueous solution of Oxone®, afforded the regioisomeric title compounds **12a–s**, possessing a *para*-methanesulfonylphenyl substituent at either the C-3 or C-4 position of the central 6-membered pyran-2-one in good yield (60–85%) as illustrated in Scheme 4.3. The 3,4,6-triphenylpyran-2-one derivatives (**12t** and **12u**) were synthesized in moderate yield (17–34%) by condensation of ethyl 4-methanesulfonylphenylacetate (**10d**) with a 1,3-diarylprop-2-yn-1-one (**9g** or **9k**) in presence of the base potassium-*t*-butoxide (Scheme 4.4).



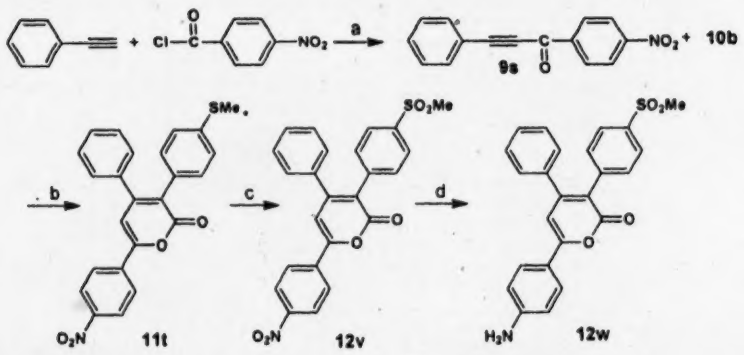
	R^1	R^2	R^3		R^1	R^2	R^3
12a	H	H	SO_2Me	**12k**	SO_2Me	H	H
12b	H	Me	SO_2Me	**12l**	SO_2Me	Me	H
12c	H	Et	SO_2Me	**12m**	SO_2Me	Et	H
12d	H	CF_3	SO_2Me	**12n**	SO_2Me	CF_3	H
12e	H	OMe	SO_2Me	**12o**	SO_2Me	OMe	H
12f	H	OEt	SO_2Me	**12p**	SO_2Me	OEt	H
12g	H	F	SO_2Me	**12q**	SO_2Me	F	H
12h	F	OMe	SO_2Me	**12r**	SO_2Me	OMe	F
12i	F	OEt	SO_2Me	**12s**	SO_2Me	OEt	F

Scheme 4.3: Reagents and conditions: (a) 1,4-dioxane, aqueous Oxone®, 25 °C, 4–5 h.



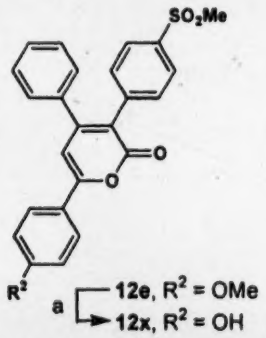
Scheme 4.4: Reagents and conditions: (a) *t*-butanol, potassium-*t*-butoxide, 50–60 °C, 1–1.5 h.

The 3,4,6-triphenylpyran-2-one **11t** with a C-6 *p*-NO_2-phenyl substituent was prepared by condensation of 1-(4-nitrophenyl)-3-phenylprop-2-yn-1-one (**9s**) with the phenylacetic acid ester **10b** in the presence of NaH. Subsequent oxidation of **11t** using aqueous Oxone® afforded the methanesulfonyl derivative **12v** in good yield (76%). The 1,3-diarylprop-2-yn-1-one (**9s**) was prepared in low yield (25%) by a copper-catalyzed cross coupling of phenylacetylene with 4-nitrobenzoyl chloride in the presence of triethylamine as base (Scheme 4.5).[26] Reduction of the nitro compound **12v** using hydrazine hydrate in the presence of Pd/C afforded the amine derivative **12w** in good yield (76%).[27] *O*-Demethylation of the C-6 methoxyphenyl compound **12e** using neat pyridinium



Scheme 4.5: Reagents and conditions: (a) Et_3N, CuI, 25 °C, 30 h (b) DMSO, NaH, 25 °C, 1 h; (c) 1,4-dioxane, aqueous Oxone®, 25 °C, 4–5 h; (d) ethanol (95%), Pd/C, $NH_2NH_2.H_2O$, 75–78 °C, 1 h.

chloride at 190–210 °C afforded the corresponding phenol derivative **12x** in low yield (20%) as shown in Scheme 4.6.[28]

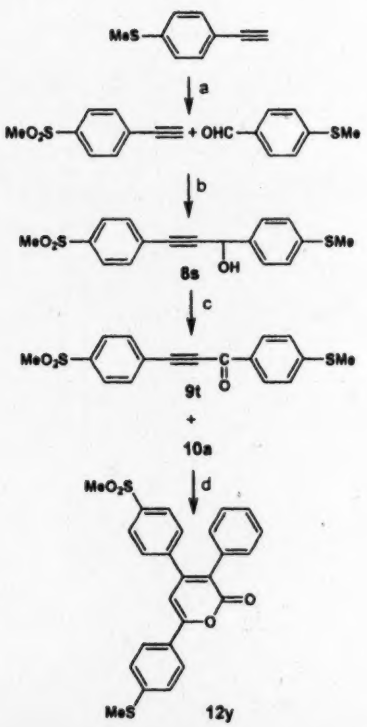


Scheme 4.6: Reagents and conditions: (a) pyridinium hydrochloride, 190–210 °C, 1–1.5 h.

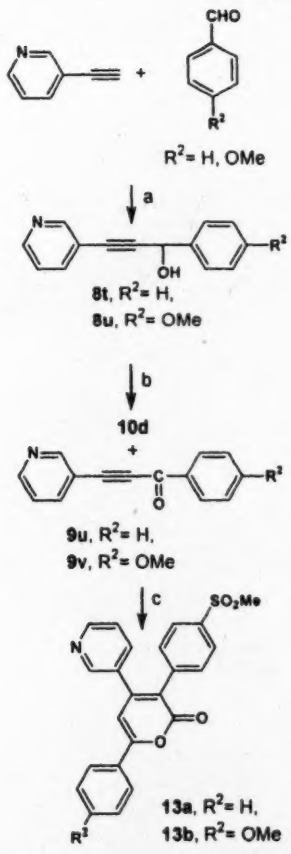
The C-4 4-methanesulfonylphenyl regioisomer **12y**, of the C-3 4-methanesulfonlyphenyl compound **12t**, was prepared using the reaction sequence shown in Scheme 4.7. Thus, the 1,3-diarylprop-2-yn-1-one derivative (**9t**) was synthesized by condensation of 1-ethynyl-4-methanesulfonylbenzene with 4-methylthiobenzaldehyde in the presence of *n*-BuLi to afford the 1,3-diarylprop-2-yn-1-ol (**8s**) which was subsequently oxidized to the corresponding ketone **9t** using activated MnO_2 in good yield (67%). The starting material 1-ethynyl-4-methanesulfonyl benzene was prepared by the oxidation of 1-ethynyl-4-methylsulfanylbenzene using aqueous Oxone® solution (Scheme 4.7). The final cyclization reaction was carried out by condensation of **9t** with ethyl phenylacetate (**10a**) in the presence of potassium-*t*-butoxide to afford the target product **12y** in good yield (46%).

The diphenylpyran-2-ones (**13a–f**), possessing a pyridyl substituent at either the C-3 or C-4 position, were prepared by the condensation of a 1,3-diarylprop-2-yn-1-one (**9u–x**) with a phenyl (pyridyl) acetic acid ester in the presence of a base as illustrated in Schemes 4.8 and 4.9.

Accordingly, reaction of 3-ethynylpyridine with a *p*-substituted-benzaldehyde (R^2 = H or OMe) in the presence of *n*-BuLi afforded the alcohol **8t** or **8u** in good yield (40–53%), which on subsequent oxidation with activated MnO_2 afforded the respective ketone **9u** or **9v** (57–61%). The C-4 pyridin-3-ylpyran-2-ones (**13a** and **13b**) were synthesized by the condensation of acetylenic ketones **9u** and **9v** with ethyl 4-methanesulfonylphenyl acetate (**10d**) in the presence of NaH in 24–46% yield as



Scheme 4.7: Reagents and conditions: (a) 1,4-dioxane, aqueous Oxone®, 25 °C, 4–5 h; (b) THF, -78 °C, *n*-BuLi, -78 °C to 25 °C over night; (c) acetone, MnO_2, 25 °C, 4–5 h; (d) *t*-butanol, potassium-*t*-butoxide, 50–60 °C, 1–1.5 h.

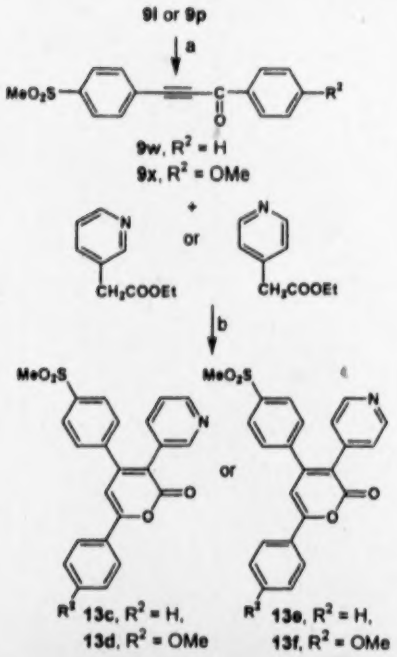


Scheme 4.8: Reagents and conditions: (a) THF, -78 °C, *n*-BuLi, -78 °C to 25 °C over night; (b) acetone, MnO_2, 25 °C, 4–5 h; (c) CH_2Cl_2, NaH, 25 °C, 1 h.

shown in Scheme 4.8. The acetylenic ketones **9l** and **9p** possessing a methylthio moiety were oxidized to the corresponding acetylenic ketones **9w** and **9x** possessing a *p*-SO_2Me substituent using aqueous Oxone® solution. Condensation of **9w** or **9x** with a pyridin-3-yl or pyridin-4-ylacetic acid ester in the presence of NaH afforded the respective C-3 pyridin-3-yl or pyridin-4-ylpyran-2-one (**13c–f**) in 22–42% yield as shown in Scheme 4.9.

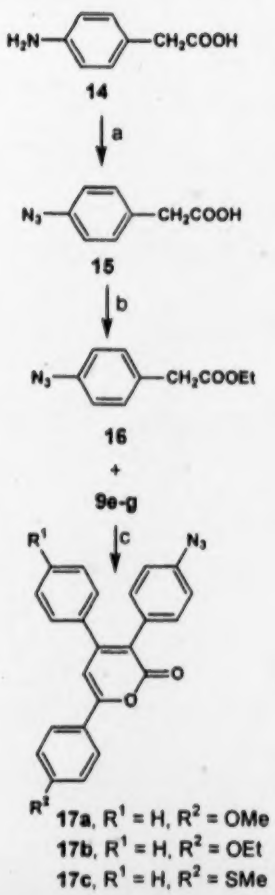
A small group of 3,4,6-triphenylpyran-2-ones in which the SO_2Me pharmacophore was replaced by a dipolar azido substituent (**17a–c**) were prepared as shown in Scheme 4.10. Accordingly, the reaction of 4-aminophenylacetic acid (**14**) with sodium nitrite under acidic conditions in the presence of sodium azide afforded 4-azidophenylacetic acid (**15**) in high yield (94%). Subsequent esterification of **15** using 95% ethanol under acidic conditions afforded the corresponding ester **16** in good yield (87%).

Condensation of the respective acetylenic ketones (**9e–g**) 4-azidophenylacetic acid



Scheme 4.9: Reagents and conditions: (a) 1,4-dioxane, aqueous Oxone®, 25 °C, 4–5 h; (b) CH_2Cl_2, NaH, 25 °C, 1 h.

ester (**16**) in the presence of NaH afforded the title compounds (**17a–c**) in moderate yield (30–35%) as shown in Scheme 4.10.

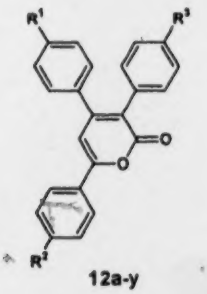


Scheme 4.10: Reagents and conditions: (a) $NaNO_2$, conc. HCl, 0 °C, 15 min, NaN_3, 15–20 min, 25 °C; (b) conc. H_2SO_4, ethanol (95%), 70–75 °C, 3–4 h; (c) CH_2Cl_2, NaH, 25 °C, 1 h.

5.3.0.0. Results and discussion

A group of regioisomeric 3,4,6-triphenylpyran-2-ones (**12a–y**, **13a–f** and **17a–c**) were designed such that the COX-2 SO_2Me pharmacophore was located at the *para*-position of either the C-3 or the C-4 phenyl substituent on the central pyran-2-one ring. In addition, the substituent at the *para*-position of the C-6 phenyl ring was varied (H, Me, Et, CF_3, OMe, OEt, F, SMe) to determine the effect of steric and electronic substituent properties on COX-2 inhibitory potency and selectivity. Structure-activity relationship (SAR) data for the title compounds (IC_{50} values) were acquired by evaluating their in vitro ability to inhibit the COX-1 and COX-2 isozymes.[19] In this regard, the pyran-2-one compounds (**12a–i** and **12t–x**) possessing a C-3 *para*-methanesulfonylphenyl substituent on the central lactone ring exhibited a broad range of COX-2 inhibitory potencies and

Table 4.1: COX-1/COX-2 Inhibitory Activities of the 3,4,6-Triphenylpyran-2-ones (**12a–y**)



12a-y

cmpd	R^1	R^2	R^3	IC_{50} (μM) [a] COX-1	IC_{50} (μM) [a] COX-2	Selectivity [b] Index (S.I.)
12a	H	H	SO_2Me	> 100	> 100	–
12b	H	Me	SO_2Me	> 100	> 100	–
12c	H	Et	SO_2Me	> 100	> 100	–
12d	H	CF_3	SO_2Me	> 100	> 100	–
12e	H	OMe	SO_2Me	> 100	0.02	> 5000
12f	H	OEt	SO_2Me	> 100	0.05	> 2000
12g	H	F	SO_2Me	> 100	5.1	> 19.0
12h	F	OMe	SO_2Me	20	1.16	17.0
12i	F	OEt	SO_2Me	3.2	158	–
12j	H	SO_2Me	H	> 100	> 100	–
12k	SO_2Me	H	H	> 100	1.3	> 77
12l	SO_2Me	Me	H	> 100	1.2	> 83
12m	SO_2Me	Et	H	> 100	1.4	71
12n	SO_2Me	CF_3	H	25.3	3.2	8.0
12o	SO_2Me	OMe	H	31.5	0.45	70
12p	SO_2Me	OEt	H	31.6	1.1	29.0
12q	SO_2Me	F	H	11.0	0.07	157
12r	SO_2Me	OMe	F	10.7	1.3	8.0
12s	SO_2Me	OEt	F	1.07	14.50	–
12t	H	SMe	SO_2Me	37.7	0.16	236
12u	F	SMe	SO_2Me	> 100	31.6	3.0
12v	H	NO_2	SO_2Me	> 100	7.0	> 14.0
12w	H	NH_2	SO_2Me	32.5	0.8	40.0
12x	H	OH	SO_2Me	> 100	> 100	–
12y	SO_2Me	SMe	H	31.2	4.5	7.0
Celecoxib				33.1	0.07	474
Rofecoxib				> 100	0.5	> 200

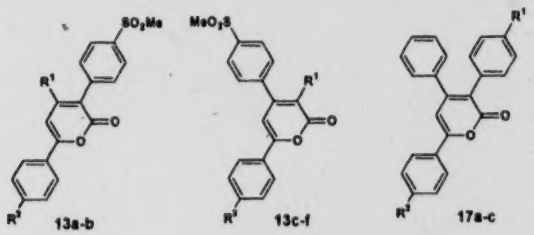
[a] Values are means of two determinations and deviation from the mean is < 10% of the mean value (Catalog No. 560101, Cayman Chemicals Inc., Ann Arbor, MI, USA).
[b] In vitro COX-2 selectivity index (IC_{50} COX-1/IC_{50} COX-2).

selectivity [IC_{50} values > 100 (inactive) to 0.02 (very potent) μM range] as summarized in Table 4.1. Varying the R^2-substituent at the *para*-position of the C-6 phenyl substituent on the central pyran-2-one had a dramatic effect on COX-2 inhibitory potency and selectivity where the C-6 *para*-methoxyphenyl compound

12e, which exhibited excellent COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.02 μM; SI > 5000), was 3.5- and 25-fold more potent than celecoxib (COX-2 IC_{50} = 0.07 μM; SI = 474) and rofecoxib (COX-2 IC_{50} = 0.50 μM; SI > 200), respectively. Insertion of a *para*-OEt substituent (**12f**) resulted in a modest decrease in COX-2 inhibitory potency (COX-2 IC_{50} = 0.05 μM). Compound **12t** possessing an electron donating R^2-thiomethyl substituent at the *para*-position of the C-6 phenyl substituent exhibited weaker COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.16 μM; COX-1 IC_{50} = 37.7 μM; SI = 236), compared to the R^2-OMe (**12e**) and R^2-OEt (**12f**) compounds. Introduction of a fluorine substituent at the *para*-position on the C-4 phenyl ring (**12h**, **12i** and **12u**) generally decreased COX-2 inhibitory potency and selectivity (Table 1). The relative COX-2 inhibitory potency order for this group of pyran-2-ones (**12a–i** and **12t–x**) was OMe > OEt > SMe > NH_2 > F > NO_2 > inactive H, Me, Et, CF_3 and OH.

The C-6 *para*-methanesulfonylphenyl regioisomer **12j** (R^2 = $MeSO_2$) was an inactive COX inhibitor (COX-1 and COX-2 IC_{50} > 100 μM). The C-4 *para*-methanesulfonylphenyl pyran-2-one

Table 4.2: COX-1/COX-2 Inhibitory Activities of 3,4,6-Triphenylpyran-2-ones (**13a–f** and **17a–c**)



cmpd	R^1	R^2	IC_{50} (μM) [a] COX-1	IC_{50} (μM) [a] COX-2	Selectivity [b] Index (S.I.)
13a	Pyridin-3-yl	H	3.0	8.0	–
13b	Pyridin-3-yl	OMe	0.44	0.03	15
13c	Pyridin-3-yl	H	3.2	0.33	10
13d	Pyridin-3-yl	OMe	0.15	1.6	–
13e	Pyridin-4-yl	H	> 100	3.0	> 33
13f	Pyridin-4-yl	OMe	> 100	0.32	> 312
17a	N_3	OMe	> 100	0.5	> 200
17b	N_3	OEt	> 100	2.3	43
17c	N_3	SMe	30.0	0.38	79
Celecoxib			33.1	0.07	474
Rofecoxib			> 100	0.5	> 200

[a] Values are means of two determinations and deviation from the mean is < 10% of the mean value (Catalog No. 560101, Cayman Chemicals Inc., Ann Arbor, MI, USA).
[b] In vitro COX-2 selectivity index (IC_{50} COX-1/IC_{50} COX-2).

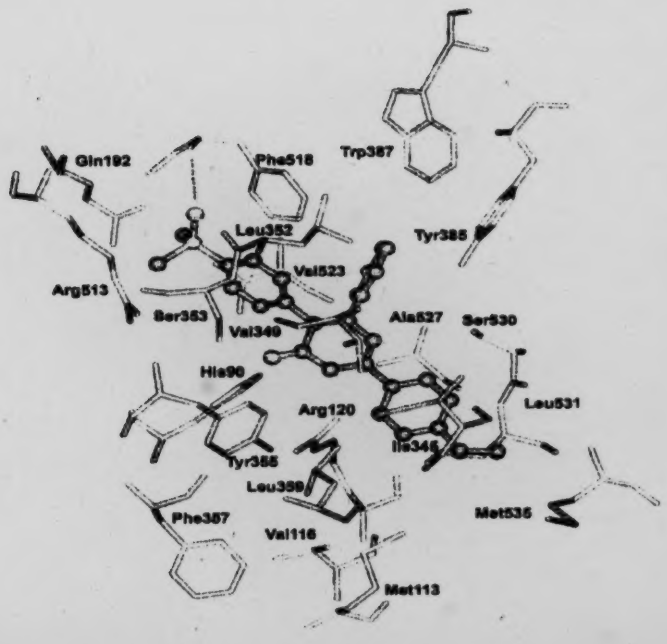


Figure 4.1: Docking 6-(4-methoxyphenyl)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (**12e**) (ball and stick) in the active site of murine COX-2 ($E_{intermolecular}$ = -90.14 kcal/mol). Red dotted line represents hydrogen bonding. Hydrogen atoms are not shown for clarity.

regioisomers (**12k–s, 12y**) exhibited moderate to good COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.07–31.6 μM range) as illustrated in Table 4.1. The C-4 *para*-methanesulfonylphenyl regioisomer **12o,** which possesses a C-6 *para*-methoxyphenyl substituent, also exhibited good COX-2 inhibitory potency (COX-2 IC_{50} = 0.45 μM; SI = 70) although it is less potent and selective than the corresponding C-3 *para*-methanesulfonylphenyl regioisomer **12e**. Introduction of a R^3-F substituent at the *para*-position of a C-3 phenyl ring significantly decreased both COX-2 inhibitory potency and selectivity (**12r** and **12s**). In contrast, the C-6 *para*-fluorophenyl compound **12q** was a highly potent COX-2 inhibitor (COX-2 IC_{50} = 0.07 μM; SI = 157) as shown in Table 4.1. In general, for the C-4 *para*-methanesulfonylphenyl group of compounds (**12k–s** and **12y**), introduction of a R^2-H, Me, Et or OEt substituent at the *para*-position of the C-6 phenyl ring provided compounds that are equipotent inhibitors of COX-2. Replacement of the C-3 or C-4 phenyl moieties of 3,4,6-triphenylpyran-2-ones by a corresponding pyridin-3-yl or pyridin-4-yl substituent significantly altered the COX-2 inhibitory potency and selectivity (Table 4.2). In this sub group, 3-(pyridin-4-yl)-4-(4-methanesulfonyl phenyl)-6-(4-methoxyphenyl)pyran-2-one (**13f**) exhibited the best combination of COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.32 μM; SI > 312).

The SO_2NH_2 and the SO_2Me pharmacophores present in celecoxib and rofecoxib respectively, are known to induce COX-2 selectivity by insertion into the secondary (2°) pocket of the COX-2 binding site that is absent in COX-1.[10] This 2°-pocket in COX-2 is formed due to a conformational change at Tyr^{355} that is attributed to the presence of Ile^{523} in COX-1 relative to Val^{523} having a smaller side chain in COX-2.[29] It has been reported that replacement of His^{513} in COX-1 by Arg^{513} in COX-2 plays a key role in the hydrogen-bond network of the COX-2 binding site.[30] Recently we exploited, for the first time, the amino acid Arg^{513} to design selective COX-2 inhibitors having a dipolar azide (N_3) pharmacophore that can undergo an electrostatic (ion-ion) interaction with Arg^{513} in the COX-2 2°-pocket.[27] Accordingly, we replaced the SO_2Me pharmacophore in the most potent and selective COX-2 inhibitors identified from the 3,4,6-triphenylpyran-2-ones investigated in this study (**12e, 12f** and **12t**) with a dipolar azido bioisostere to evaluate compounds **17a–c** (see Table 4.2). It is biologically relevant that compounds possessing a dipolar *p*-N_3 pharmacophore on the C-3 phenyl substituent (**17a–c**) retained their COX-2 selectivity, even though they are less potent (COX-2 IC_{50} = 0.38–2.3 μM

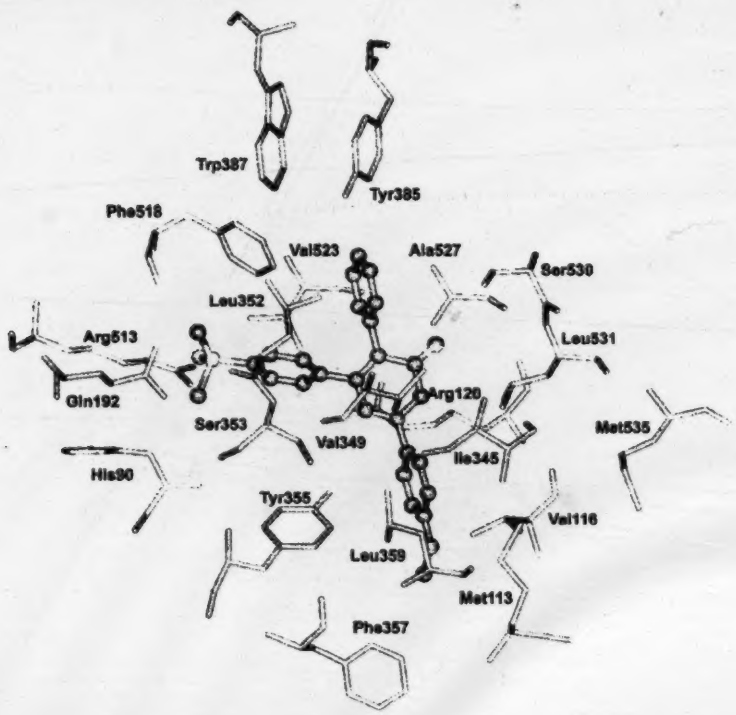


Figure 4.2: Docking 6-(4-methoxyphenyl)-4-(4-methanesulfonylphenyl)-3-phenylpyran-2-one (**12o**) (ball and stick) in the active site of murine COX-2 ($E_{intermolecular}$ = -84.44 kcal/mol). Hydrogen atoms are not shown for clarity.

range; SI = 79 to > 200 range) than the corresponding C-3 *p*-$MeSO_2$-phenyl analogs.

The orientation and binding interactions of the potent and selective COX-2 inhibitor **12e** [6-(4-methoxyphenyl)-3-(4-methanesulfonyl phenyl)-4-phenylpyran-2-one] within the COX-2 active site was investigated by a molecular modeling (docking) experiment (Figure 4.1). The C-3 *para*-methanesulfonylphenyl regioisomer **12e** binds in the center of the COX-2 binding site such that the SO_2Me pharmacophore inserts deep into the COX-2 secondary pocket, and the *para*-methanesulfonyl phenyl moiety of the ligand which undergoes hydrophobic contact with the protein, is surrounded by Phe^{518}, Arg^{513}, Gln^{192}, Ser^{353}, Leu^{352} and Val^{523}. Due to the presence of a less bulky Val^{523} in COX-2, relative to Ile^{523} in COX-1, the *S*-atom of the SO_2Me substituent is positioned about 4.53 Å inside the entrance to the COX-2 secondary pocket with one of its oxygen atoms forming a hydrogen bond with the N*H* of Phe^{518} (distance = 2.19 Å). The distance between the other SO_2Me oxygen atom and the NH_2 (guanidino group) of Arg^{513} is about 3.71 Å. The C-4 unsubstituted phenyl ring was oriented towards a hydrophobic pocket comprised of Trp^{387}, Tyr^{385}, Leu^{384} and Tyr^{348} at the top of the COX-2 binding site. The distance between the center of the C-4 phenyl ring and *O*H of Ser^{530} was about 6.15 Å. The central 6-membered lactone (pyran-2-one) ring was located near the mouth of the COX-2 binding site such that, the central C=*O* of the lactone ring undergoes a weak hydrogen bonding interaction with the O*H* of Ser^{353} (distance = 2.91 Å). The interspacial distance between the O*H* of Tyr^{355} and C=*O* is about 5.11 Å, and the *O*-atom of the central lactone ring is positioned about 3.26 Å from the NH_2 (guanidino group) of Arg^{120}.

The C-6 *para*-methoxyphenyl substituent of **12e** is oriented towards a hydrophobic pocket close to the mouth of the COX-2 active site such that it is within a van der Waal's contact range (distance ≈ 5 Å) of Ala^{527}, Ser^{530}, Leu^{531}, Met^{535}, Ile^{345}, Val^{349}, and Leu^{359}. The distance between the center of the C-6 phenyl ring and the *O*H of Ser^{530} was about 4.86 Å. The phenyl ring (of the C-6 *p*-MeO-phenyl substituent) undergoes a cation-π interaction with the NH_2 of the guanidino side chain of Arg^{120} (distance = 6.0 Å). This interaction may confer important COX-2 selectivity implications by disrupting the salt bridge between Arg^{120} and Glu^{524} at the mouth of the COX-2 binding site.[29,31] Interestingly, the *p*-OMe substituent attached to the C-6 phenyl ring is within a van der Waal's contact range of Met^{535} (distance ≈ 5 Å), and the OMe group interacts favorably with the -SMe side chain of Met^{535}.

A similar docking study for the C-4 *p*-$MeSO_2$-phenyl regioisomer **12o** in the COX-2 binding site shows that the C-4 *para*-methanesulfonylphenyl moiety is positioned in the vicinity of amino acid residues (Phe^{518}, Arg^{513} and Gln^{192}) lining the COX-2 secondary pocket as shown in Figure 4.2. Unlike the C-3 regioisomer **12e**, the *O*-atom of the SO_2Me group in **12o** is not hydrogen bonded to Phe^{518} (distance = 3.10 Å), and the methanesulfonyl *S*-atom is located about 3.8 Å inside the entrance to the COX-2 secondary pocket (Val^{523}). Similar to the C-3 regioisomer **12e**, the C-3 unsubstituted-phenyl ring in **12o** is oriented towards a hydrophobic pocket made up of Trp^{387}, Tyr^{385} and Tyr^{348}. The

C=*O* of the central lactone ring is about 4.33 Å away from the O*H* of Ser[530]. The C-6 *p*-MeO-phenyl moiety of **12o** is located near the mouth of the COX-2 binding site, and it is surrounded by amino acid residues Met[113], Val[116], Arg[120], Val[349], Tyr[355], Phe[357] and Leu[359], and the OMe substituent is much further removed from Met[535] compared to the C-3 *p*-$MeSO_2$-phenyl regioisomer **12e**.

Conformational comparisons of the binding modes of the two regioisomers **12e** and **12o** in the COX-2 active site are shown in Figure 4.3. The root mean square deviation (RMSD) between these two conformations was ~ 0.04 Å. The atom pairs of the vicinal diaryl system were selected for superimposition of **12e** and **12o** (the lactone C-3, C-4 and the

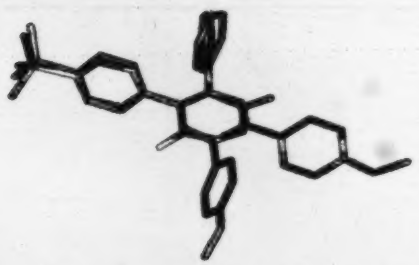


Figure 4. 3: Overlay of the binding modes of **12e** (blue) and **12o** (green) within the active site of murine COX-2. Hydrogen atoms are not shown for clarity.

aromatic carbons C-1, C'-1). The vicinal aromatic rings of both **12e** and **12o,** and also their *p*-sulfonyl group, lie in a common plane. However, the SO_2Me group of **12e** extends much deeper inside the COX-2 secondary pocket relative to **12o** (the *S*-atom of the SO_2Me substituent of **12e** is positioned about 4.53 Å inside the entrance to the COX-2 secondary pocket unlike **12o**). The central 6-membered pyran-2-one ring of both **12e** and **12o** lie in a common plane. It is noteworthy that due to the regioisomeric position of the SO_2Me group, on either the C-3 or C-4 phenyl ring of the central lactone ring, the C=O is either close to Tyr[355] (in **12e**) at the entrance to COX-2 secondary pocket or near Ser[530] (in **12o**) within the COX-2 binding site. Recent studies have shown the important interaction of diarylheterocyclic selective COX-2 inhibitors with Tyr[355].[32] In addition, the C-6 *p*-MeO-phenyl substituent in the two regioisomers is positioned in different regions within the COX-2 binding site. In this regard, interaction of the *p*-MeO-phenyl ring substituent of **12e** within a hydrophobic pocket comprised of Ile[345], Val[349], Leu[359] and Met[535] appears to orient the C-3 *p*-$MeSO_2$-phenyl substituent deeper into the COX-2 secondary pocket which does not occur in the case of the C-4 *p*-$MeSO_2$-phenyl regioisomer **12o**. A series of molecular dynamics (MD) simulations on the stabilities of the enzyme-ligand complexes reveal that **12e** ($E_{intermolecular}$ = -90.14 kcal/mol) has a higher binding affinity for COX-2 as compared to **12o** ($E_{intermolecular}$ = -84.44 kcal/mol). This data is consistent with the more potent COX-2 inhibition exhibited by **12e** (COX-2 IC_{50} = 0.02; S.I. > 5000) as compared to **12o** (COX-2 IC_{50} = 0.45; S.I. = 70).

The binding mode of the pyran-2-one **17a**, in which the COX-2 SO_2Me pharmacophore was replaced by a linear azido (N_3) bioisostere, within the COX-2 active site is similar to that of the respective C-3 *para*-$MeSO_2$-phenyl analog **12e**, although there are some subtle differences (Figure 4.4). As expected, the linear dipolar azido substituent of the C-3 *p*-N_3-phenyl moiety is oriented towards the COX-2 secondary pocket with the dipolar N_3

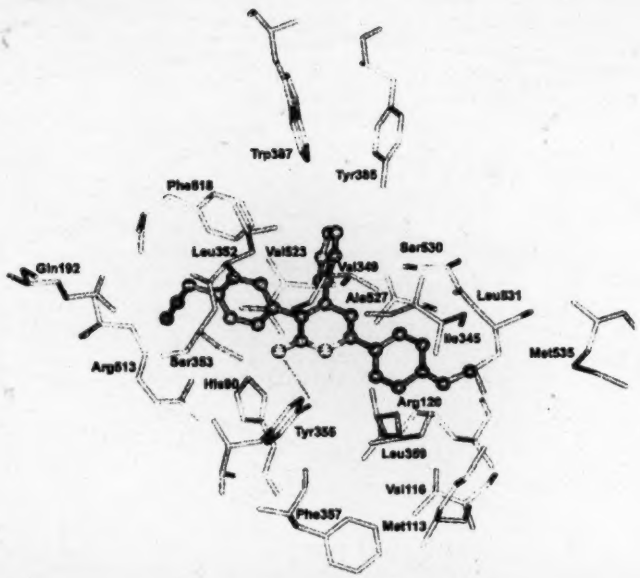
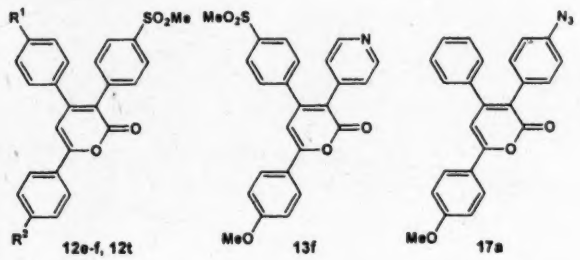


Figure 4.4: Docking 6-(4-methoxyphenyl)-3-(4-azidophenyl)-4-phenylpyran-2-one (**17a**) (ball and stick) in the active site of murine COX-2 ($E_{intermolecular}$ = -77.48 kcal/mol). Red dotted line represents hydrogen bonding. Hydrogen atoms are not shown for clarity.

group participating in favourable interactions (electrostatic) with Arg^{513} and Phe^{518}.[27] The terminal *N*-atom of the dipolar azide group is located about 3.23 Å inside the COX-2 secondary pocket (Val^{523}), and about 2.63 Å removed from the NH_2 (guanidino group) of Arg^{513} (ion-ion interaction). The terminal *N*-atom can also undergo a favourable interaction with the backbone N*H* of Phe^{518} (distance = 3.81 Å). In addition, the terminal *N*-atom of the dipolar azide group is nearly 4.65 Å from the N*H* of His^{90} at the entrance to the COX-2 secondary pocket. The C-4 unsubstituted phenyl ring is located near a hydrophobic pocket comprised of Trp^{387}, Tyr^{385} and Leu^{384} observed similar to the related SO_2Me analog **12e**. The central C=*O* of the lactone ring is hydrogen bonded to the O*H* of Tyr^{355} (distance = 2.50 Å) at the mouth of the COX-2 binding site. The C-6 *p*-MeO-phenyl ring is within van der Waal's contact range (distance = 5 Å) with the protein, and it is surrounded by Ile^{345}, Leu^{531}, Leu^{359} and Met^{535}. The C-6 phenyl ring also undergoes a cation-π interaction with the NH_2 of the guanidino side chain of Arg^{120} (distance = 6.13 Å). These observations show that the dipolar azido pharmacophore, serves as a suitable bioisostere that undergoes electrostatic interaction with Arg^{513} within the COX-2 secondary pocket. Accordingly, COX-2 selectivity is retained for compounds belonging to the 3,4,6-triphenylpyran-2-one class by replacing the traditional SO_2Me pharmacophore by a linear dipolar azido substituent (**17a**, COX-2 IC_{50} = 0.50 μM; S.I. > 200).

Our previous study on C-3 *para*-methanesulfonylphenyl pyranones indicated that C-6 alkyl-, alkoxy- or alkylthio- substituents are a major determinant of COX-2 inhibitory potency

Table 4.3: Antiinflammatory and Analgesic Activities of 3,4,6-Triphenylpyran-2-ones (**12e–f, 12t, 13f** and **17a**)



cmpd	R^1	R^2	AI Activity [a] % Inhibition at 3 hours	Analgesic Activity [b] % Inhibition at 30 min.	% Inhibition at 60 min.
12e	H	OMe	31.0 ± 4.7 [c]	37.5 ± 14.4	69.0 ± 14.0
12f	H	OEt	61.3 ± 1.2 [d]	58.3 ± 10.2	75.0 ± 17.6
12t	H	SMe	52.6 ± 1.6 [e]	72.0 ± 12.3	64.7 ± 19.4
13f			50.3 ± 8.0 [f,g]	58.3 ± 8.3	55.0 ± 9.1
17a			42.3 ± 7.6 [h]	47.2 ± 10.0	55.5 ± 13.6
Ibuprofen			56.2 ± 2.0 [i]	–	–
Celecoxib			80.0 ± 2.0 [j,k]	69.33 ± 12.1	79.5 ± 2.0

[a] Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as mean ± SEM (n = 4–6) following a 5 mg/kg oral dose of the test compound.
[b] Inhibitory activity in the rat 4% NaCl-induced abdominal constriction assay. The results are expressed as mean ± SEM (n = 4–6) following a 5 mg/kg oral dose of the test compound.
[c] ID_{50} = 19.2 mg/kg oral dose. [d] ID_{50} = 5.6 mg/kg oral dose. [e] ID_{50} = 7.9 mg/kg oral dose.
[f] ID_{50} = 21.4 mg/kg oral dose. [g] 30 mg/kg oral dose. [h] ID_{50} = 5.6 mg/kg oral dose. [i] 50 mg/kg oral dose.
[j] 50 mg/kg oral dose. [k] ID_{50} = 10.8 mg/kg oral dose.

and selectivity due to their ability to orient the central pyranone ring such that the C-3 *para*-SO_2Me pharmacophore is positioned in the vicinity of the COX-2 secondary pocket.[19] In this study on 3,4,6-triphenylpyranones, it is shown that a C-6 *p*-substituted-phenyl ring also plays a critical role in positioning the *para*-SO_2Me pharmacophore close to the COX-2 secondary pocket. In addition, it appears that the regioisomeric placement of the *para*-SO_2Me pharmacophore at either the C-3 or C-4 phenyl ring in this latter group of C-6 phenyl analogs is an important determinant with respect to COX-2 inhibitory potency and selectivity. In this regard, the C-3 *p*-SO_2Me substituted pyranones (**12e**, **12f** and **12t**) exhibited superior COX-2 inhibitory potency and selectivity.

Pharmacological studies were carried out to assess the in vivo antiinflammatory

and analgesic activity of some of the most potent and selective COX-2 inhibitors (**12e**, **12f**, **12t**, **13f** and **17a**) based on in vitro enzyme inhibition data (Table 4.3). In a carrageenan-induced rat paw edema assay model, **12e** (COX-2 IC_{50} = 0.02; S.I. > 5000) exhibited a 31% inhibition of inflammation at 3 h after administration of a 5 mg/kg oral dose. The most active oral antiinflammatory compound **12f** (COX-2 IC_{50} = 0.05; S.I. > 2000) that exhibited an ID_{50} of 5.6 mg/kg was more potent than the reference drug celecoxib (ID_{50} = 10.8 mg/kg). In a rat model 4% NaCl-induced abdominal constriction assay, a 5 mg/kg po dose of these pyran-2-ones exhibited good analgesic activities (37–75% range) at 30 or 60 minutes post drug administration.

The 6-(4-ethoxyphenyl)-3-(4-methane sulfonylphenyl)-4-phenylpyran-2-one (**12f**) exhibited good analgesic activity where a 5 mg/kg po dose reduced writhing by 58 and 75% at 30 and 60 min post drug administration.

5.4.0.0. Conclusions

The SAR data acquired for this class of 3,4,6-triphenylpyran-2-ones have shown that (i) compounds exhibiting high COX-2 inhibitory potency and selectivity can be designed by appropriate placement of a *p*-SO_2Me pharmacophore on either a C-3 or C-4 phenyl ring, in which the pyran-2-one ring serves as a suitable central ring template, (ii) docking studies revealed that the C-3 *p*-$MeSO_2$-phenyl regioisomer having an appropriately substituted C-6 phenyl ring, exhibit better binding affinity than the corresponding C-4 *p*-$MeSO_2$-phenyl regioisomer, (iii) COX-2 inhibitory potency and selectivity is sensitive to substituent electronic properties at the *para*-position of the C-6 phenyl ring where the C-6 *p*-MeO-phenyl compound **12e** exhibits the best combination of potency and selectivity, and (iv) the linear azido (N_3) substituent is a suitable bioisostere to replace a traditional SO_2Me COX-2 pharmacophore.

5.5.0.0. Experimental Section

General. Melting points were determined using a Buchi capillary apparatus and are uncorrected. Ibuprofen was purchased from Sigma (St. Louis, MO). All other reagents including **8a** (1,3-diphenylprop-2-yn-1-ol) and **9a** (1,3-diphenylprop-2-yn-1-one) were purchased from Aldrich (Milwaukee, WI). Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70-230 mesh). Infrared (IR) spectra were recorded using a Nicolet 550 Series II Magna FT-IR spectrometer. Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were recorded on a Bruker AM-300 spectrometer and chemical shifts are expressed in parts per million (ppm, δ) relative to tetramethylsilane as internal standard. Spin multiplets are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) and m (multiplet). Coupling constants (*J*) are given in Hertz (Hz). ^{13}C NMR spectra were acquired using the *J* modulated spin echo technique where methyl and methine carbons appear as positive peaks and methylene and quaternary carbon resonances appear as negative peaks. Microanalyses were performed for C, H and N (Micro Analytical Service Laboratory, Department of Chemistry, University of Alberta) and were within ± 0.4% of the theoretical values. Celecoxib and rofecoxib were synthesized

according to the literature procedures[11,12] Compounds **8b** [1-(4-methylphenyl)-3-phenylprop-2-yn-1-ol], **8e** [1-(4-methoxylphenyl)-3-phenylprop-2-yn-1-ol], **8h** [1-(4-fluorophenyl)-3-phenylprop-2-yn-1-ol], **9b** [1-(4-methylphenyl)-3-phenylprop-2-yn-1-one], **9e** [1-(4-methoxyphenyl)-3-phenylprop-2-yn-1-one], 1-ethynyl-4-methanesulfonylbenzene and the phenylacetic acid esters (**10b** and **10c**) were prepared according to the previously reported methods.[33-37] Male Sprague-Dawley rats, used in the antinflammatory-analgesic screens, were purchased from Animal Health Services at the University of Alberta, and experiments were carried out using protocols approved by the Animal Welfare Committee, University of Alberta.

General Procedure for the Synthesis of 1,3-Diarylprop-2-yn-1-ols (8c–r). A 4-substituted-phenylacetylene (**6**, R^1 = H, SMe or F; 9.75 mmol) was added slowly under an argon atmosphere to a stirred solution of freshly dried THF (10 mL) at –78 °C. A solution of *n*-BuLi (4 mL of 2.5 M in hexane) was added slowly. After 3 minutes a solution of the respective 4-substituted-benzaldehyde (**7**, R^2 = H, Me, Et, CF_3, OMe, OEt, SMe or F; 9.75 mmol) in dry THF (5 mL) was added slowly while maintaining the temperature at –78 °C, and the reaction was allowed to proceed overnight after stirring and warming to room temperature. The reaction mixture was washed with saturated aqueous NH_4Cl (10 mL), extracted with EtOAc (2 x 20 mL), the organic phase was separated, dried over Na_2SO_4, and the solvent was evaporated in vacuo to give a crude oil which was purified by silica gel column chromatography using hexane–ethyl acetate (3:1, v/v) as eluent to afford the respective title compound **8c–r** in 44–84% yield. Some physical and spectroscopic data for **8c–r** are listed below.

1-(4-Ethylphenyl)-3-phenylprop-2-yn-1-ol (8c). The product was obtained as a white solid (1.4 g, 61%): mp 108–110 °C; IR (film): 3315 (OH), 2193 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.22 (t, *J* = 7.3 Hz, 3H, CH_2CH_3), 2.22 (d, *J* = 5.8 Hz, 1H, CHO*H*), 2.63 (q, *J* = 7.3 Hz, 2H, CH_2CH_3), 5.66 (d, *J* = 5.8 Hz, 1H, C*H*OH), 7.23 (d, *J* = 8.2 Hz, 2H, 4-ethylphenyl H-3, H-5), 7.28–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.42–7.49 (m, 2H, phenyl H-2, H-6), 7.52 (d, *J* = 8.2 Hz, 2H, 4-ethylphenyl H-2, H-6). Anal. ($C_{17}H_{16}O$): C, H.

1-(4-Trifluoromethylphenyl)-3-phenylprop-2-yn-1-ol (8d). The product was obtained as a pale yellow oil (1.5 g, 59%): IR (film): 3313 (OH), 2183 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.40 (d, *J* = 5.8 Hz, 1H, CHO*H*), 5.64 (d, *J* = 5.8 Hz, 1H, C*H*OH), 7.30–7.35 (m, 3H, phenyl H-3, H-4, H-5), 7.44–7.49 (m, 2H, phenyl H-2, H-6), 7.66 (d, *J* = 8.2 Hz, 2H, 4-trifluorophenyl H-2, H-6), 7.74 (d, *J* = 8.2 Hz, 2H, 4-trifluorophenyl H-3, H-5).

1-(4-Ethoxyphenyl)-3-phenylprop-2-yn-1-ol (8f). The product was obtained as a pale yellow oil (1.6 g, 65%): IR (film): 3310 (OH), 2188 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.40 (t, *J* = 7.3 Hz, 3H, OCH_2CH_3), 2.19 (d, *J* = 5.8 Hz, 1H, CHO*H*), 4.07 (q, *J* = 7.3 Hz, 2H, OCH_2CH_3), 5.64 (d, *J* = 5.8 Hz, 1H, C*H*OH), 6.91 (d, *J* = 8.5 Hz, 2H, 4-ethoxyphenyl H-3, H-5), 7.30–7.45 (m,

3H, phenyl H-3, H-4, H-5), 7.47–7.51 (m, 2H, phenyl H-2, H-6), 7.53 (d, J = 8.5 Hz, 2H, 4-ethoxyphenyl H-2, H-6). Anal. ($C_{17}H_{16}O_2$): C, H.

1-(4-Methylsulfanylphenyl)-3-phenylprop-2-yn-1-ol (8g). The product was obtained as a pale yellow solid (1.95 g, 74.5%): mp 56–58 °C; IR (film): 3310 (OH), 2188 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.24 (d, J = 5.8 Hz, 1H, CHO*H*), 2.50 (s, 3H, SCH_3), 5.60 (d, J = 5.8 Hz, 1H, C*H*OH), 7.28 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.32–7.35 (m, 3H, phenyl H-3, H-4, H-5), 7.44–7.49 (m, 2H, phenyl H-2, H-6), 7.53 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-2, H-6). Anal. ($C_{16}H_{14}OS$): C, H.

3-(4-Fluorophenyl)-1-(4-methoxyphenyl)prop-2-yn-1-ol (8i). The product was obtained as an oil (2.11 g, 84.4%): IR (film): 3320 (OH), 2124 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.19 (d, J = 5.8 Hz, 1H, CHO*H*), 3.87 (s, 3H, OCH_3), 5.63 (d, J = 5.8 Hz, 1H, C*H*OH), 6.96 (d, J = 8.5 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.00 (dd, J^3_{HH} = 8.2, J^3_{FH} = 8.2 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.43 (dd, J^3_{HH} = 8.2, J^4_{FH} = 4.9 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.52 (d, J = 8.5 Hz, 2H, 4-methoxyphenyl H-2, H-6). Anal. ($C_{16}H_{13}FO_2$): C, H.

3-(4-Fluorophenyl)-1-(4-ethoxyphenyl)prop-2-yn-1-ol (8j). The product was obtained as an oil (2.06 g, 77.7%): IR (film): 3374 (OH), 2120 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.41 (t, J = 7.3 Hz, 3H, OCH_2CH_3), 2.16 (d, J = 5.8 Hz, 1H, CHO*H*), 4.07 (q, J = 7.3 Hz, 2H, OCH_2CH_3), 5.62 (d, J = 5.8 Hz, 1H, C*H*OH), 6.90 (d, J = 8.5 Hz, 2H, 4-ethoxyphenyl H-3, H-5), 6.97 (dd, J^3_{HH} = 8.2, J^3_{FH} = 8.2 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.42 (dd, J^3_{HH} = 8.2, J^4_{FH} = 4.9 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.51 (d, J = 8.5 Hz, 2H, 4-ethoxyphenyl H-2, H-6). Anal. ($C_{17}H_{15}FO_2$): C, H.

3-(4-Fluorophenyl)-1-(4-methylsulfanylphenyl)prop-2-yn-1-ol (8k). The product was obtained as an oil (1.78 g, 67.2%): IR (film): 3347 (OH), 2135 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.22 (d, J = 5.8 Hz, 1H, CHO*H*), 2.50 (s, 3H, SCH_3), 5.64 (d, J = 5.8 Hz, 1H, C*H*OH), 6.98 (dd, J^3_{HH} = 8.2, J^3_{FH} = 8.2 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.25 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.41 (dd, J^3_{HH} = 8.2, J^4_{FH} = 4.9 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.51 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-2, H-6). Anal. ($C_{16}H_{13}FOS$): C, H.

3-(4-Methylsulfanylphenyl)-1-phenylprop-2-yn-1-ol (8l). The product was obtained as a reddish brown oil (1.98 g, 80%): IR (film): 3335 (OH), 2226 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.26 (d, J = 6.1 Hz, 1H, CHO*H*), 2.48 (s, 3H, SCH_3), 5.68 (d, J = 6.1 Hz, 1H, C*H*OH), 7.16 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.32 (d, J = 8.5 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.36–7.44 (m, 3H, phenyl H-3, H-4, H-5), 7.60–7.63 (m, 2H, phenyl H-2, H-6). Anal. ($C_{16}H_{14}OS$): C, H.

3-(4-Methylsulfanylphenyl)-1-(4-methylphenyl)prop-2-yn-1-ol (8m). The product was obtained as an oil (1.63 g, 62.2%): IR (film): 3351 (OH), 2220 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.19 (d, J = 5.8 Hz, 1H, CHO*H*), 2.38 (s, 3H, CH_3), 2.49 (s, 3H, SCH_3), 5.65 (d, J = 5.8 Hz, 1H, C*H*OH), 7.16 (d, J = 8.8 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.21

(d, J = 8.0 Hz, 2H, 4-methylphenyl H-3, H-5), 7.37 (d, J = 8.8 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.40 (d, J = 8.0 Hz, 2H, 4-methylphenyl H-2, H-6). Anal. ($C_{17}H_{16}OS$): C, H.

3-(4-Methylsulfanylphenyl)-1-(4-ethylphenyl)prop-2-yn-1-ol (8n). The product was obtained as an oil (1.96 g, 71.2%): IR (film): 3324 (OH), 2193 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.22 (t, J = 7.6 Hz, 3H, CH_2CH_3), 2.18 (d, J = 6.1 Hz, 1H, CHO*H*), 2.49 (s, 3H, SCH_3), 2.53 (q, J = 7.6 Hz, 2H, CH_2CH_3), 5.65 (d, J = 6.1 Hz, 1H, C*H*OH), 7.15 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.17 (d, J = 8.2 Hz, 2H, 4-ethylphenyl H-3, H-5), 7.37 (d, J = 8.2 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.52 (d, J = 8.2 Hz, 2H, 4-ethylphenyl H-2, H-6). Anal. ($C_{18}H_{18}OS$): C, H.

3-(4-Methylsulfanylphenyl)-1-(4-trifluoromethylphenyl)prop-2-yn-1-ol (8o). The product was obtained as an oil (1.35 g, 43%): IR (film): 3355 (OH), 2227 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.40 (d, J = 5.8 Hz, 1H, CHO*H*), 2.49 (s, 3H, SCH_3), 5.74 (d, J = 5.8 Hz, 1H, C*H*OH), 7.17 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.36 (d, J = 8.5 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.66 (d, J = 8.2 Hz, 2H, 4-trifluoromethylphenyl H-2, H-6), 7.73 (d, J = 8.2 Hz, 2H, 4-trifluoromethylphenyl H-3, H-5). Anal. ($C_{17}H_{13}F_3OS$): C, H.

3-(4-Methylsulfanylphenyl)-1-(4-methoxyphenyl)prop-2-yn-1-ol (8p). The product was obtained as an oil (2.32 g, 83.7%): IR (film): 3351 (OH), 2220 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.17 (d, J = 6.1 Hz, 1H, CHO*H*), 2.49 (s, 3H, SCH_3), 3.81 (s, 3H, OCH_3), 5.64 (d, J = 6.1 Hz, 1H, C*H*OH), 6.92 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.16 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.37 (d, J = 8.5 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.53 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-2, H-6). Anal. ($C_{17}H_{16}O_2S$): C, H.

3-(4-Methylsulfanylphenyl)-1-(4-ethoxyphenyl)prop-2-yn-1-ol (8q). The product was obtained as a dark brown oil (2.14 g, 73.5%): IR (film): 3328 (OH), 2206 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.40 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.17 (d, J = 5.8 Hz, 1H, CHO*H*), 2.49 (s, 3H, SCH_3), 4.02 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 5.65 (d, J = 5.8 Hz, 1H, C*H*OH), 6.91 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-3, H-5), 7.16 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.37 (d, J = 8.2 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.51 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-2, H-6). Anal. ($C_{18}H_{18}O_2S$): C, H.

3-(4-Methylsulfanylphenyl)-1-(4-fluorophenyl)prop-2-yn-1-ol (8r). The product was obtained as an oil (1.96 g, 73.8%): IR (film): 3382 (OH), 2220 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.28 (d, J = 6.1 Hz, 1H, CHO*H*), 2.49 (s, 3H, SCH_3), 5.66 (d, J = 6.1 Hz, 1H, C*H*OH), 7.09 (dd, J^3_{HH} = 8.8, J^3_{FH} = 8.8 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.17 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.36 (d, J = 8.2 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.56 (dd, J^3_{HH} = 8.8, J^4_{FH} = 5.5 Hz, 2H, 4-fluorophenyl H-2, H-6). Anal. ($C_{16}H_{13}FOS$): C, H.

General Procedure for the Synthesis of 1,3-Diarylprop-2-yn-1-ones (9c–r). To a stirred solution of the respective 1,3-diarylprop-2-yn-1-ol (**8c–r**; 4.5 mmol) in acetone (25 mL) was added activated

manganese IV oxide (7.8 g, 90 mmol), and the reaction mixture was stirred for 4–5 h at 25 °C after which MnO_2 was filtered off, and the organic solvent was removed in vacuo to give the title compound (**9c–r**) in good yield (52–85%). Some physical and spectroscopic data for **9c–r** are listed below.

1-(4-Ethylphenyl)-3-phenylprop-2-yn-1-one (9c). The product was obtained as a oil by the oxidation of **8c** in the presence of MnO_2 (0.74 g, 70.2%): IR (film): 2152 (C≡C), 1650 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.25 (t, J = 7.3 Hz, 3H, CH_2CH_3), 2.71 (q, J = 7.3 Hz, 2H, CH_2CH_3), 7.33 (d, J = 8.2 Hz, 2H, 4-ethylphenyl H-3, H-5), 7.35–7.51 (m, 3H, phenyl H-3, H-4, H-5), 7.67–7.69 (m, 2H, phenyl H-2, H-6), 8.13 (d, J = 8.2 Hz, 2H, 4-ethylphenyl H-2, H-6). Anal. ($C_{17}H_{14}O$): C, H.

1-(4-Trifluoromethylphenyl)-3-phenylprop-2-yn-1-one (9d). The product was obtained as a white solid by the oxidation of **8d** in the presence of MnO_2 (0.96 g, 77.8%): mp 70–72 °C; IR (film): 2150 (C≡C), 1649 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.43–7.56 (m, 3H, phenyl H-3, H-4, H-5), 7.67–7.73 (m, 2H, phenyl H-2, H-6), 7.79 (d, J = 8.2 Hz, 2H, 4-trifluoromethylphenyl H-3, H-5), 8.33 (d, J = 8.2 Hz, 2H, 4-trifluoromethylphenyl H-2, H-6). Anal. ($C_{16}H_9F_3O$): C, H.

1-(4-Ethoxyphenyl)-3-phenylprop-2-yn-1-one (9f). The product was obtained as a white solid by the oxidation of **8f** in the presence of MnO_2 (1.3 g, 53%): mp 57–59 °C; IR (film): 2152 (C≡C), 1645 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.49 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 4.11 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.90 (d, J = 8.5 Hz, 2H, 4-ethoxyphenyl H-3, H-5), 7.39–7.51 (m, 3H, phenyl H-3, H-4, H-5), 7.67–7.69 (m, 2H, phenyl H-2, H-6), 8.18 (d, J = 8.5 Hz, 2H, 4-ethoxyphenyl H-2, H-6).

1-(4-Methylsulfanylphenyl)-3-phenylprop-2-yn-1-one (9g). The product was obtained as a solid by the oxidation of **8g** in the presence of MnO_2 (0.95 g, 83.6%): mp 50–51 °C; IR (film): 2172 (C≡C), 1642 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.54 (s, 3H, SCH_3), 7.31 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.40–7.51 (m, 3H, phenyl H-3, H-4, H-5), 7.61–7.67 (m, 2H, phenyl H-2, H-6), 8.11 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-2, H-6). Anal. ($C_{16}H_{12}OS$): C, H.

1-(4-Fluorophenyl)-3-phenylprop-2-yn-1-one (9h). The product was obtained as a solid by the oxidation of **8h** in the presence of MnO_2 (1.4 g, 63%): mp 61–63 °C; IR (film): 2200 (C≡C), 1649 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.16 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.40–7.50 (m, 3H, phenyl H-3, H-4, H-5), 7.67–7.70 (m, 2H, phenyl H-2, H-6), 8.26 (dd, J^3_{HH} = 8.5, J^4_{FH} = 4.9 Hz, 2H, 4-fluorophenyl H-2, H-6).

3-(4-Fluorophenyl)-1-(4-methoxyphenyl)prop-2-yn-1-one (9i). The product was obtained as a solid by the oxidation of **8i** in the presence of MnO_2 (0.61 g, 53.3%): mp 104–106 °C; IR (film): 2193 (C≡C), 1632 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.90 (s, 3H, OCH_3), 6.98 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.00 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.43 (dd, J^3_{HH} = 8.5, J^4_{FH} = 4.9 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.52 (d, J = 8.5

Hz, 2H, 4-methoxyphenyl H-2, H-6). Anal. ($C_{16}H_{11}FO_2$): C, H.

3-(4-Fluorophenyl)-1-(4-ethoxyphenyl)prop-2-yn-1-one (9j). The product was obtained as a solid by the oxidation of **8j** in the presence of MnO_2 (0.68 g, 56.5%): mp 81–83 °C; IR (film): 2193 (C≡C), 1622 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.41 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 4.07 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.96 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-3, H-5), 7.09 (dd, J^3_{HH} = 8.8, J^3_{FH} = 8.8 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.65 (dd, J^3_{HH} = 8.8, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 8.16 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-2, H-6). Anal. ($C_{17}H_{13}FO_2$): C, H.

3-(4-Fluorophenyl)-1-(4-methylsulfanylphenyl)prop-2-yn-1-one (9k). The product was obtained as a solid by the oxidation of **8k** in the presence of MnO_2 (0.76 g, 62.4%): mp 119–121 °C; IR (film): 2206 (C≡C), 1635 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.53 (s, 3H, SCH_3), 7.13 (dd, J^3_{HH} = 8.2, J^3_{FH} = 8.2 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.33 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.66 (dd, J^3_{HH} = 8.2, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 8.10 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-2, H-6). Anal. ($C_{16}H_{11}FOS$): C, H.

3-(4-Methylsulfanylphenyl)-1-phenylprop-2-yn-1-one (9l). The product was obtained as a solid by the oxidation of **8l** in the presence of MnO_2 (0.77 g, 68%): mp 54–55 °C; IR (film): 2200 (C≡C), 1629 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.52 (s, 3H, SCH_3), 7.23 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.50 (d, J = 8.5 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.54–7.66 (m, 3H, phenyl H-3, H-4, H-5), 8.20–8.23 (m, 2H, phenyl H-2, H-6). Anal. ($C_{16}H_{12}OS$): C, H.

3-(4-Methylsulfanylphenyl)-1-(4-methylphenyl)prop-2-yn-1-one (9m). The product was obtained as an oil by the oxidation of **8m** in the presence of MnO_2 (0.66 g, 55%): IR (film): 2186 (C≡C), 1616 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.38 (s, 3H, CH_3), 2.53 (s, 3H, SCH_3), 7.24 (d, J = 8.0 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.30 (d, J = 8.2 Hz, 2H, 4-methylphenyl H-3, H-5), 7.58 (d, J = 8.0 Hz, 2H, methylsulfanylphenyl H-2, H-6), 8.10 (d, J = 8.2 Hz, 2H, 4-methylphenyl H-2, H-6). Anal. ($C_{17}H_{14}OS$): C, H.

3-(4-Methylsulfanylphenyl)-1-(4-ethylphenyl)prop-2-yn-1-one (9n). The product was obtained as a solid by the oxidation of **8n** in the presence of MnO_2 (0.62 g, 49%): mp 45–46 °C; IR (film): 2193 (C≡C), 1635 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.22 (t, J = 7.6 Hz, 3H, CH_2CH_3), 2.53 (s, 3H, SCH_3), 2.71 (q, J = 7.6 Hz, 2H, CH_2CH_3), 7.17 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.24 (d, J = 8.2 Hz, 2H, 4-ethylphenyl H-3, H-5), 7.58 (d, J = 8.5 Hz, 2H, methylsulfanylphenyl H-2, H-6), 8.12 (d, J = 8.2 Hz, 2H, 4-ethylphenyl H-2, H-6). Anal. ($C_{18}H_{16}OS$): C, H.

3-(4-Methylsulfanylphenyl)-1-(4-trifluoromethylphenyl)prop-2-yn-1-one (9o). The product was obtained as a solid by the oxidation of **8o** in the presence of MnO_2 (0.82 g, 57.2%): mp 88–90 °C; IR (film): 2220 (C≡C), 1636 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.54 (s, 3H, SCH_3), 7.26 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.59 (d, J = 8.2 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.78 (d, J = 8.2 Hz, 2H, 4-

trifluoromethylphenyl H-3, H-5), 8.31 (d, J = 8.2 Hz, 2H, 4-trifluoromethylphenyl H-2, H-6). Anal. ($C_{17}H_{11}F_3OS$): C, H.

3-(4-Methylsulfanylphenyl)-1-(4-methoxyphenyl)prop-2-yn-1-one (9p). The product was obtained as a solid by the oxidation of **8p** in the presence of MnO_2 (0.86 g, 67.8%): mp 80–82 °C; IR (film): 2200 (C≡C), 1618 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.52 (s, 3H, SCH_3), 3.88 (s, 3H, OCH_3), 6.97 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.23 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.56 (d, J = 8.5 Hz, 2H, methylsulfanylphenyl H-2, H-6), 8.17 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-2, H-6). Anal. ($C_{17}H_{14}O_2S$): C, H.

3-(4-Methylsulfanylphenyl)-1-(4-ethoxyphenyl)prop-2-yn-1-one (9q). The product was obtained as a oil by the oxidation of **8q** in the presence of MnO_2 (0.97 g, 67.5%): IR (film): 2220 (C≡C), 1636 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.47 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.53 (s, 3H, SCH_3), 4.10 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.96 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-3, H-5), 7.23 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.57 (d, J = 8.5 Hz, 2H, methylsulfanylphenyl H-2, H-6), 8.16 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-2, H-6). Anal. ($C_{18}H_{16}O_2S$): C, H.

3-(4-Methylsulfanylphenyl)-1-(4-fluorophenyl)prop-2-yn-1-one (9r). The product was obtained as a solid by the oxidation of **8r** in the presence of MnO_2 (0.54 g, 44.7%): mp 99–100 °C; IR (film): 2193 (C≡C), 1630 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.52 (s, 3H, SCH_3), 7.16 (dd, J^3_{HH} = 8.8, J^3_{FH} = 8.8 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.26 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.57 (d, J = 8.5 Hz, 2H, methylsulfanylphenyl H-2, H-6), 8.20 (dd, J^3_{HH} = 8.8, J^4_{FH} = 5.5 Hz, 2H, 4-fluorophenyl H-2, H-6). Anal. ($C_{16}H_{11}FOS$): C, H.

General Procedure for the Synthesis of 3,4,6-Triphenylpyran-2-ones (11a–s). To a stirred solution of the ethyl 4-substituted-phenylacetate (**10a–c**, R^3 = H, SMe or F; 1.7 mmol) in DMSO (10 mL) was added NaH (95% dry powder, 1.9 mmol) immediately after which, the respective 1,3-diarylprop-2-yn-1-one (**9a–r**, 1.7 mmol) in DMSO (10 mL) was added slowly. The reaction mixture was stirred at 25 °C for 1 h after which it was washed with 1N HCl (10 mL), extracted with EtOAc (2 x 20 mL), the organic phase was separated, dried over Na_2SO_4, and the organic portion was evaporated in vacuo. The brownish oil obtained was purified by silica gel column chromatography using hexane–ethyl acetate (1:2, v/v or 1:3, v/v) as eluent to afford the respective title compound 11a–s in 22–62% yield. Some physical and spectroscopic data for **11a–s** are listed below.

3-(4-Methylsulfanylphenyl)-4,6-diphenylpyran-2-one (11a). The product was obtained as a yellow solid by condensation of **9a** with **10b** in the presence of NaH (0.34 g, 55%): mp 232–234 °C; IR (film): 1703 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.45 (s, 3H, SCH_3), 6.84 (s, 1H, pyranone H-5), 7.09 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.12 (d, J = 8.2 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.18–7.21 (m, 2H, phenyl H-2, H-6), 7.29–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.47–7.49 (m, 3H, phenyl H-3, H-4, H-5),

7.89–7.93 (m, 2H, phenyl H-2, H-6). Anal. ($C_{24}H_{18}O_2S$): C, H.

6-(4-Methylphenyl)-3-(4-methylsulfanylphenyl)-4-phenylpyran-2-one (11b). The product was obtained as a yellow solid by condensation of **9b** with **10b** in the presence of NaH (0.18 g, 28.3%): mp 246–248 °C; IR (film): 1705 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.42 (s, 3H, CH_3), 2.45 (s, 3H, SCH_3), 6.79 (s, 1H, pyranone H-5), 7.08 (d, J = 9.0 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.11 (d, J = 9.0 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.15–7.17 (m, 2H, phenyl H-2, H-6), 7.20–7.23 (m, 3H, phenyl H-3, H-4, H-5), 7.28 (d, J = 8.5 Hz, 2H, 4-methylphenyl H-3, H-5), 7.79 (d, J = 8.5 Hz, 2H, 4-methylphenyl H-2, H-6). Anal. ($C_{25}H_{20}O_2S$): C, H.

6-(4-Ethylphenyl)-3-(4-methylsulfanylphenyl)-4-phenylpyran-2-one (11c). The product was obtained as a yellow solid by condensation of **9c** with **10b** in the presence of NaH (0.23 g, 34.5%): mp 216–217 °C; IR (film): 1709 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.28 (t, J = 7.6 Hz, 3H, CH_2CH_3), 2.45 (s, 3H, SCH_3), 2.68 (q, J = 7.6 Hz, 2H, CH_2CH_3), 6.80 (s, 1H, pyranone H-5), 7.08 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.12 (d, J = 8.2 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.14–7.20 (m, 2H, phenyl H-2, H-6), 7.23–7.26 (m, 3H, phenyl H-3, H-4, H-5), 7.28 (d, J = 8.2 Hz, 2H, 4-ethylphenyl H-3, H-5), 7.81 (d, J = 8.2 Hz, 2H, 4-ethylphenyl H-2, H-6). Anal. ($C_{26}H_{22}O_2S$): C, H.

3-(4-Methylsulfanylphenyl)-4-phenyl-6-(4-trifluoromethylphenyl)pyran-2-one (11d). The product was obtained as a yellow oil by condensation of **9d** with **10b** in the presence of NaH (0.22 g, 30.3%): IR (film): 1735 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.46 (s, 3H, SCH_3), 6.91 (s, 1H, pyranone H-5), 7.09 (d, J = 8.8 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.13 (d, J = 8.8 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.19–7.21 (m, 2H, phenyl H-2, H-6), 7.26–7.32 (m, 3H, phenyl H-3, H-4, H-5), 7.72 (d, J = 8.2 Hz, 2H, 4-trifluoromethylphenyl H-2, H-6), 8.0 (d, J = 8.2 Hz, 2H, 4-trifluoromethylphenyl H-3, H-5). Anal. ($C_{25}H_{17}F_3O_2S$): C, H.

6-(4-Methoxyphenyl)-3-(4-methylsulfanylphenyl)-4-phenylpyran-2-one (11e). The product was obtained as a oil by condensation of **9e** with **10b** in the presence of NaH (0.23 g, 34.4%): IR (film): 1703 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.45 (s, 3H, SCH_3), 3.87 (s, 3H, OCH_3), 6.72 (s, 1H, pyranone H-5), 6.97 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.09 (d, J = 8.0 Hz, 2H, methylsulfanylphenyl H-3, H-5), 7.11 (d, J = 8.0 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.16–7.18 (m, 2H, phenyl H-2, H-6), 7.20–7.23 (m, 3H, phenyl H-3, H-4, H-5), 7.85 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-2, H-6). Anal. ($C_{25}H_{20}O_3S$): C, H.

6-(4-Ethoxyphenyl)-3-(4-methylsulfanylphenyl)-4-phenylpyran-2-one (11f). The product was obtained as a yellow solid by condensation of **9f** with **10b** in the presence of NaH (0.15 g, 22.2%): mp 212–213 °C; IR (film): 1709 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.43 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.45 (s, 3H, SCH_3), 4.10 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.72 (s, 1H, pyranone H-5), 6.95 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-3, H-5), 7.11 (d, J = 8.2 Hz, 2H, 4-

methylsulfanylphenyl H-3, H-5), 7.13 (d, J = 8.2 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.16–7.20 (m, 2H, phenyl H-2, H-6), 7.23–7.30 (m, 3H, phenyl H-3, H-4, H-5), 7.83 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-2, H-6). Anal. ($C_{26}H_{22}O_3S$): C, H.

6-(4-Fluorophenyl)-3-(4-methylsulfanylphenyl)-4-phenylpyran-2-one (11g). The product was obtained as a yellow solid by condensation of **9h** with **10b** in the presence of NaH (0.28 g, 43.3%): mp 235–237 °C; IR (film): 1703 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.45 (s, 3H, SCH_3), 6.77 (s, 1H, pyranone H-5), 7.09 (d, J = 8.8 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.12 (d, J = 8.8 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.14 (dd, J^3_{HH} = 8.2, J^3_{FH} = 8.2 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.18–7.20 (m, 2H, phenyl H-2, H-6), 7.25–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.88 (dd, J^3_{HH} = 8.2, J^4_{FH} = 4.9 Hz, 2H, 4-fluorophenyl H-2, H-6). Anal. ($C_{24}H_{17}FO_2S$): C, H.

4-(4-Fluorophenyl)-6-(4-methoxyphenyl)-3-(4-methylsulfanylphenyl)pyran-2-one (11h). The product was obtained as a yellow solid by condensation of **9i** with **10b** in the presence of NaH (0.40 g, 57%): mp 196–198 °C; IR (film): 1712 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.46 (s, 3H, SCH_3), 3.87 (s, 3H, OCH_3), 6.68 (s, 1H, pyranone H-5), 6.95 (dd, J^3_{HH} = 8.4, J^3_{FH} = 8.4 Hz, 2H, 4-fluorophenyl H-3, H-5), 6.97 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.08 (d, J = 9.0 Hz, 2H, methylsulfanylphenyl H-3, H-5), 7.11 (d, J = 9.0 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.13 (dd, J^3_{HH} = 8.4, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.84 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-2, H-6). Anal. ($C_{25}H_{19}FO_3S$): C, H.

6-(4-Ethoxyphenyl)-4-(4-fluorophenyl)3-(4-methylsulfanylphenyl)pyran-2-one (11i). The product was obtained as a yellowish oil by condensation of **9j** with **10b** in the presence of NaH (0.33 g, 44.8%): IR (film): 1698 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.44 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.46 (s, 3H, SCH_3), 3.99 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.60 (s, 1H, pyranone H-5), 6.85 (dd, J^3_{HH} = 8.4, J^3_{FH} = 8.4 Hz, 2H, 4-fluorophenyl H-3, H-5), 6.90 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-3, H-5), 7.03 (d, J = 9.0 Hz, 2H, methylsulfanylphenyl H-3, H-5), 7.09 (d, J = 9.0 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.10 (dd, J^3_{HH} = 8.4, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.75 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-2, H-6). Anal. ($C_{26}H_{21}FO_3S$): C, H.

6-(4-Methylsulfanylphenyl)-3,4-diphenylpyran-2-one (11j). The product was obtained as a yellow solid by condensation of **9g** with **10a** in the presence of NaH (0.28 g, 45%): mp 170–172°C; IR (film): 1708 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.55 (s, 3H, SCH_3), 6.80 (s, 1H, pyranone H-5), 7.15–7.27 (m, 10H, phenyl H-2, H-3, H-4, H-5, H-6), 7.28 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.81 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-2, H-6). Anal. ($C_{24}H_{18}O_2S$): C, H.

4-(4-Methylsulfanylphenyl)-3,6-diphenylpyran-2-one (11k). The product was obtained as a yellow solid by condensation of **9l** with **10a** in the presence of NaH (0.33g, 52.5%): mp 177–179 °C; IR (film): 1709 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.45 (s, 3H, SCH_3),

6.83 (s, 1H, pyranone H-5), 7.07 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.09 (d, J = 8.2 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.20–7.29 (m, 5H, phenyl H-2, H-3, H-4, H-5, H-6), 7.47–7.49 (m, 3H, phenyl H-3, H-4, H-5), 7.89–7.93 (m, 2H, phenyl H-2, H-6). Anal. ($C_{24}H_{18}O_2S$): C, H.

6-(4-Methylphenyl)-4-(4-methylsulfanylphenyl)-3-phenylpyran-2-one (11l). The product was obtained as a yellow solid by condensation of **9m** with **10a** in the presence of NaH (0.27 g, 41.5%): mp 150–152 °C; IR (film): 1709 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.43 (s, 3H, CH_3), 2.46 (s, 3H, SCH_3), 6.78 (s, 1H, pyranone H-5), 7.08 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.12 (d, J = 8.0 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.14–7.20 (m, 2H, phenyl H-2, H-6), 7.23–7.26 (m, 3H, phenyl H-3, H-4, H-5), 7.28 (d, J = 8.2 Hz, 2H, 4-methylphenyl H-3, H-5), 7.79 (d, J = 8.2 Hz, 2H, 4-methylphenyl H-2, H-6). Anal. ($C_{25}H_{20}O_2S$): C, H.

6-(4-Ethylphenyl)-4-(4-methylsulfanylphenyl)-3-phenylpyran-2-one (11m). The product was obtained as a solid by condensation of **9n** with **10a** in the presence of NaH (0.30 g, 44.8%): mp 128–130 °C; IR (film): 1709 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.26 (t, J = 7.6 Hz, 3H, CH_2CH_3), 2.46 (s, 3H, SCH_3), 2.68 (q, J = 7.6 Hz, 2H, CH_2CH_3), 6.78 (s, 1H, pyranone H-5), 7.06 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.10 (d, J = 8.2 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.19–7.26 (m, 5H, phenyl H-2, H-3, H-4, H-5, H-6), 7.29 (d, J = 8.2 Hz, 2H, 4-ethylphenyl H-3, H-5), 7.81 (d, J = 8.2 Hz, 2H, 4-ethylphenyl H-2, H-6). Anal. ($C_{26}H_{22}O_2S$): C, H.

4-(4-Methylsulfanylphenyl)-3-phenyl-6-(4-trifluoromethylphenyl)-pyran-2-one (11n). The product was obtained as a yellow oil by condensation of **9o** with **10a** in the presence of NaH (0.39 g, 52.3%): IR (film): 1703 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.47 (s, 3H, SCH_3), 6.90 (s, 1H, pyranone H-5), 7.08 (d, J = 8.8 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.13 (d, J = 8.8 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.20–7.30 (m, 5H, phenyl H-2, H-3, H-4, H-5, H-6), 7.73 (d, J = 8.5 Hz, 2H, 4-trifluoromethylphenyl H-2, H-6), 8.01 (d, J = 8.5 Hz, 2H, 4-trifluoromethylphenyl H-3, H-5). Anal. ($C_{25}H_{17}F_3O_2S$): C, H.

6-(4-Methoxyphenyl)-4-(4-methylsulfanylphenyl)-3-phenylpyran-2-one (11o). The product was obtained as a yellow solid by condensation of **9p** with **10a** in the presence of NaH (0.25 g, 37.7%): mp 156–158 °C; IR (film): 1710 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.46 (s, 3H, SCH_3), 3.88 (s, 3H, OCH_3), 6.71 (s, 1H, pyranone H-5), 6.97 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.08 (d, J = 8.8 Hz, 2H, methylsulfanylphenyl H-3, H-5), 7.11 (d, J = 8.8 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.19–7.32 (m, 5H, phenyl H-2, H-3, H-4, H-5, H-6), 7.85 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-2, H-6). Anal. ($C_{25}H_{20}O_3S$): C, H.

6-(4-Ethoxyphenyl)-4-(4-methylsulfanylphenyl)-3-phenylpyran-2-one (11p). The product was obtained as a yellow solid by condensation of **9q** with **10a** in the presence of NaH (0.44 g, 62.4%): mp 152–154 °C; IR (film): 1712 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.43 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.46 (s, 3H, SCH_3), 4.07 (q, J = 7.0 Hz, 2H,

OCH_2CH_3), 6.70 (s, 1H, pyranone H-5), 6.95 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-3, H-5), 7.09 (d, J = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.11 (d, J = 8.2 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.19–7.29 (m, 5H, phenyl H-2, H-3, H-4, H-5, H-6), 7.83 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-2, H-6). Anal. ($C_{26}H_{22}O_3S$): C, H.

6-(4-Fluorophenyl)-4-(4-methylsulfanylphenyl)-3-phenylpyran-2-one (11q). The product was obtained as a yellow solid by condensation of **9r** with **10a** in the presence of NaH (0.26 g, 39.4%): mp 93–95 °C; IR (film): 1708 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.46 (s, 3H, SCH_3), 6.75 (s, 1H, pyranone H-5), 7.09 (d, J = 8.8 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.12 (d, J = 8.8 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.14 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.19–7.30 (m, 5H, phenyl H-2, H-3, H-4, H-5, H-6), 7.88 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6). Anal. ($C_{24}H_{17}FO_2S$): C, H.

3-(4-Fluorophenyl)-6-(4-methoxyphenyl)-4-(4-methylsulfanylphenyl)pyran-2-one (11r). The product was obtained as a yellow solid by condensation of **9p** with **10c** in the presence of NaH (0.40 g, 56%): mp 156–158 °C; IR (film): 1712 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.46 (s, 3H, SCH_3), 3.87 (s, 3H, OCH_3), 6.70 (s, 1H, pyranone H-5), 6.91–7.00 (m, 4H, fluorophenyl H-3, H-5; 4-methoxyphenyl H-3, H-5), 7.05 (d, J = 9.0 Hz, 2H, methylsulfanylphenyl H-3, H-5), 7.08 (d, J = 9.0 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.13 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.84 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-2, H-6). Anal. ($C_{25}H_{19}FO_3S$): C, H.

6-(4-Ethoxyphenyl)-3-(4-fluorophenyl)-4-(4-methylsulfanylphenyl)pyran-2-one (11s). The product was obtained as a yellow solid by condensation of **9q** with **10c** in the presence of NaH (0.36 g, 49.4%): mp 160–162 °C; IR (film): 1698 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.43 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.47 (s, 3H, SCH_3), 4.07 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.69 (s, 1H, pyranone H-5), 6.92–7.01 (m, 4H, fluorophenyl H-3, H-5; 4-ethoxyphenyl H-3, H-5), 7.05 (d, J = 8.5 Hz, 2H, methylsulfanylphenyl H-3, H-5), 7.08 (d, J = 8.5 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.11 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.83 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-2, H-6). Anal. ($C_{26}H_{21}FO_3S$): C, H.

General Procedure for the Synthesis of 3,4,6-Triphenylpyran-2-ones (12a–s). An aqueous solution of Oxone® (50% w/v , 1.62 mmol) was added drop wise to a stirred solution of a 3,4,6-triphenylpyran-2-one (**11a–s**, 0.54 mmol) possessing a 4-methylsulfanyl substituent on either the C-3, C-4 or C-6 phenyl ring in 1,4-dioxane (10 mL) at 0 °C. The reaction was allowed to proceed with stirring at 25 °C for 4–5 h. The reaction mixture was diluted with water (10 mL), extracted with EtOAc (2 x 20 mL), the EtOAc fraction was washed successively with brine solution and water (10 mL each), the organic phase was separated, dried over Na_2SO_4, and the solvent was removed in vacuo to give a crude oil. This oil was purified by silica gel column chromatography using hexane–ethyl acetate (1:2, v/v or 1:3, v/v) as eluent to

afford the respective title compound **12a–s** in 60–85% yield. Some physical and spectroscopic data for **12a–s** are listed below.

3-(4-Methanesulfonylphenyl)-4,6-diphenylpyran-2-one (12a). The product was obtained as a yellow solid by oxidation of **11a** in the presence of aqueous Oxone® solution (0.14 g, 65%): mp 278–280 °C; IR (film): 1709 (C=O), 1306, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.04 (s, 3H, SO_2CH_3), 6.89 (s, 1H, pyranone H-5), 7.12–7.16 (m, 2H, phenyl H-2, H-6), 7.28–7.37 (m, 3H, phenyl H-3, H-4, H-5), 7.40 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.44–7.51 (m, 3H, phenyl H-3, H-4, H-5), 7.82 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5), 7.90–7.93 (m, 2H, phenyl H-2, H-6). Anal. ($C_{24}H_{18}O_4S$): C, H.

6-(4-Methylphenyl)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (12b). The product was obtained as a yellow solid by oxidation of **11b** in the presence of aqueous Oxone® solution (0.18 g, 82%): mp 224–226 °C; IR (film): 1703 (C=O), 1320, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 2.96 (s, 3H, SO_2CH_3), 6.77 (s, 1H, pyranone H-5), 7.04–7.08 (m, 2H, phenyl H-2, H-6), 7.18–7.29 (m, 5H, phenyl H-3, H-4, H-5; 4-methylphenyl H-3, H-5), 7.32 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.71 (d, J = 8.5 Hz, 2H, 4-methylphenyl H-2, H-6), 7.73 (d, J = 8.2 Hz, 2H, methanesulfonylphenyl H-3, H-5). Anal. ($C_{25}H_{20}O_4S$): C, H.

6-(4-Ethylphenyl)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (12c). The product was obtained as a oil by oxidation of **11c** in the presence of aqueous Oxone® solution (0.21 g, 92.5%): IR (film): 1705 (C=O), 1306, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.24 (t, J = 7.6 Hz, 3H, CH_2CH_3), 2.69 (q, J = 7.6 Hz, 2H, CH_2CH_3), 3.03 (s, 3H, SO_2CH_3), 6.85 (s, 1H, pyranone H-5), 7.11–7.15 (m, 2H, phenyl H-2, H-6), 7.26–7.38 (m, 5H, phenyl H-3, H-4, H-5; 4-ethylphenyl H-3, H-5), 7.40 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.79 (d, J = 8.5 Hz, 2H, 4-ethylphenyl H-2, H-6), 7.83 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5). Anal. ($C_{26}H_{22}O_4S$): C, H.

3-(4-Methanesulfonylphenyl)-4-phenyl-6-(4-trifluoromethylphenyl)-pyran-2-one (12d). The product was obtained as a yellowish oil by oxidation of **11d** in the presence of aqueous Oxone® solution (0.17 g, 67%): IR (film): 1735 (C=O), 1315, 1142 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.04 (s, 3H, SO_2CH_3), 6.96 (s, 1H, pyranone H-5), 7.13–7.16 (m, 2H, phenyl H-2, H-6), 7.24–7.39 (m, 3H, phenyl H-3, H-4, H-5), 7.41 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.75 (d, J = 8.2 Hz, 2H, 4-trifluoromethylphenyl H-2, H-6), 7.81 (d, J = 8.2 Hz, 2H, methanesulfonylphenyl H-3, H-5), 8.03 (d, J = 8.2 Hz, 2H, 4-trifluoromethylphenyl H-3, H-5). Anal. ($C_{25}H_{17}F_3O_4S$): C, H.

6-(4-Methoxyphenyl)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (12e). The product was obtained as a yellow solid by oxidation of **11e** in the presence of aqueous Oxone® solution (0.20 g, 86.2%): mp 218–220 °C; IR (film): 1705 (C=O), 1315, 1157 (SO_2) cm^{-1}; ^1H NMR

($CDCl_3$): δ 3.03 (s, 3H, SO_2CH_3), 3.89 (s, 3H, OCH_3), 6.77 (s, 1H, pyranone H-5), 6.99 (d, J = 8.2 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.12–7.16 (m, 2H, phenyl H-2, H-6), 7.22–7.38 (m, 3H, phenyl H-3, H-4, H-5), 7.40 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, J = 8.2 Hz, 2H, 4-methoxyphenyl H-2, H-6), 7.87 (d, J = 8.2 Hz, 2H, methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 44.5 (SO_2CH_3), 55.5 (OCH_3), 103.5 (pyranone C-5), 114.4 (4-methoxyphenyl C-3, C-5), 119.5 (pyranone C-3), 123.1 (4-methoxyphenyl C-1), 126.9 (phenyl C-3, C-5), 128.5, 128.5 and 128.7 (4-methoxyphenyl C-2, C-6; 4-methanesulfonylphenyl C-2, C-6; phenyl C-2, C-6), 129.2 (phenyl C-4), 131.9 (4-methanesulfonylphenyl C-3, C-5), 137.0 (phenyl C-1), 139.0 (4-methanesulfonylphenyl C-1), 140.1 (4-methanesulfonylphenyl C-4), 154.7 (pyranone C-4), 159.5 (4-methoxyphenyl C-4), 161.9 (pyranone C-6), 162.1 (pyranone C-2). Anal. ($C_{25}H_{20}O_5S$): C, H.

6-(4-Ethoxyphenyl)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (12f). The product was obtained as a yellow solid by oxidation of **11f** in the presence of aqueous Oxone® solution (0.18 g, 78.5%): mp 250–252 °C; IR (film): 1698 (C=O), 1313, 1142 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.44 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 3.03 (s, 3H, SO_2CH_3), 4.08 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.77 (s, 1H, pyranone H-5), 6.97 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-3, H-5), 7.11–7.14 (m, 2H, phenyl H-2, H-6), 7.25–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.39 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.78 (d, J = 8.2 Hz, 2H, 4-ethoxyphenyl H-2, H-6), 7.85 (d, J = 8.2 Hz, 2H, methanesulfonylphenyl H-3, H-5); ^{13}C NMR ($CDCl_3$): δ 14.7 (OCH_2CH_3), 44.5 (SO_2CH_3), 63.8 (OCH_2CH_3), 103.4 (pyranone C-5), 114.9 (4-ethoxyphenyl C-3, C-5), 119.4 (pyranone C-3), 123.2 (4-ethoxyphenyl C-1), 126.9 (phenyl C-3, C-5), 128.5, 128.5 and 128.7 (4-ethoxyphenyl C-2, C-6; 4-methanesulfonylphenyl C-2, C-6; phenyl C-2, C-6), 129.2 (phenyl C-4), 132.0 (4-methanesulfonylphenyl C-3, C-5), 137.0 (phenyl C-1), 139.0 (4-methanesulfonylphenyl C-1), 140.1 (4-methanesulfonylphenyl C-4), 154.7 (pyranone C-4), 159.6 (4-ethoxyphenyl C-4), 161.5 (pyranone C-6), 162.1 (pyranone C-2). Anal. ($C_{26}H_{22}O_5S$): C, H.

6-(4-Fluorophenyl)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (12g). The product was obtained as a yellow solid by oxidation of **11g** in the presence of aqueous Oxone® solution (0.18 g, 79.6%): mp 294–296 °C; IR (film): 1709 (C=O), 1320, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.04 (s, 3H, SO_2CH_3), 6.82 (s, 1H, pyranone H-5), 7.12–7.14 (m, 2H, phenyl H-2, H-6), 7.15 (dd, J^3_{HH} = 8.2, J^3_{FH} = 8.2 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.20–7.35 (m, 3H, phenyl H-3, H-4, H-5), 7.40 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-2, H-6), 7.80 (d, J = 8.2 Hz, 4-methanesulfonylphenyl H-3, H-5), 7.91 (dd, J^3_{HH} = 8.2, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6). Anal. ($C_{24}H_{17}FO_4S$): C, H.

4-(4-Fluorophenyl)-6-(4-methoxyphenyl)-3-(4-methanesulfonylphenyl)pyran-2-one (12h). The product was obtained as a yellow solid by oxidation of **11h** in the presence of aqueous Oxone® solution (0.21 g, 88%): mp 224–226 °C; IR (film): 1709 (C=O), 1315, 1158 (SO_2) cm^{-1}; ^1H

NMR ($CDCl_3$): δ 3.04 (s, 3H, SO_2CH_3), 3.88 (s, 3H, OCH_3), 6.73 (s, 1H, pyranone H-5), 6.95 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 6.97 (d, J = 8.2 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.09 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.39 (d, J = 8.8 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.81 (d, J = 8.2 Hz, 2H, 4-methoxyphenyl H-2, H-6), 7.86 (d, J = 8.8 Hz, 2H, methanesulfonylphenyl H-3, H-5). Anal. ($C_{25}H_{19}FO_5S$): C, H.

4-(4-Fluorophenyl)-6-(4-ethoxyphenyl)-3-(4-methanesulfonylphenyl)pyran-2-one (12i). The product was obtained as a yellow solid by oxidation of **11i** in the presence of aqueous Oxone® solution (0.19 g, 75.7%): mp 243–245 °C; IR (film): 1703 (C=O), 1310, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.45 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 2.98 (s, 3H, SO_2CH_3), 4.00 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.64 (s, 1H, pyranone H-5), 6.88 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 6.90 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-3, H-5), 7.07 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.31 (d, J = 9.0 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.73 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-2, H-6), 7.77 (d, J = 9.0 Hz, 2H, methanesulfonylphenyl H-3, H-5). Anal. ($C_{26}H_{21}FO_5S$): C, H.

6-(4-Methanesulfonylphenyl)-3,4-diphenylpyran-2-one (12j). The product was obtained as a yellow solid by oxidation of **11j** in the presence of aqueous Oxone® solution (0.18 g, 82.8%): mp 178–180 °C; IR (film): 1716 (C=O), 1306, 1159 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.12 (s, 3H, SO_2CH_3), 6.97 (s, 1H, pyranone H-5), 7.16–7.39 (m, 10H, phenyl H-2, H-3, H-4, H-5, H-6), 8.07 (d, J = 8.8 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 8.10 (d, J = 8.8 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{24}H_{18}O_4S$): C, H.

4-(4-Methanesulfonylphenyl)-3,6-diphenylpyran-2-one (12k). The product was obtained as a yellow solid by oxidation of **11k** in the presence of aqueous Oxone® solution (0.16 g, 76.3%): mp 188–190 °C; IR (film): 1723 (C=O), 1315, 1157 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.05 (s, 3H, SO_2CH_3), 6.78 (s, 1H, pyranone H-5), 7.15–7.18 (m, 2H, phenyl H-2, H-6), 7.21–7.26 (m, 3H, phenyl H-3, H-4, H-5), 7.37 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.48–7.52 (m, 3H, phenyl H-3, H-4, H-5), 7.86 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5), 7.90–7.93 (m, 2H, phenyl H-2, H-6). Anal. ($C_{24}H_{18}O_4S$): C, H.

6-(4-Methylphenyl)-4-(4-methanesulfonylphenyl)-3-phenylpyran-2-one (12l). The product was obtained as a oil by oxidation of **11l** in presence of aqueous Oxone® solution (0.17g, 78.7%): IR (film): 1695 (C=O), 1317, 1148 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.44 (s, 3H, CH_3), 3.05 (s, 3H, SO_2CH_3), 6.74 (s, 1H, pyranone H-5), 7.12–7.17 (m, 2H, phenyl H-2, H-6), 7.18–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.34 (d, J = 8.4 Hz, 2H, 4-methylphenyl H-3, H-5), 7.37 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.79 (d, J = 8.2 Hz, 2H, 4-methylphenyl H-2, H-6), 7.82 (d, J = 8.2 Hz, 2H, methanesulfonylphenyl H-3, H-5). Anal. ($C_{25}H_{20}O_4S$): C, H.

6-(4-Ethylphenyl)-4-(4-methanesulfonylphenyl)-3-phenylpyran-2-one (12m). The product was obtained as a yellow solid by oxidation of **11m** in the presence of aqueous Oxone® solution (0.19 g, 85%): mp 173–175 °C; IR (film): 1709 (C=O), 1315, 1157 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.26 (t, J = 7.6 Hz, 3H, CH_2CH_3), 3.05 (s, 3H, SO_2CH_3), 2.71 (q, J = 7.6 Hz, 2H, CH_2CH_3), 6.74 (s, 1H, pyranone H-5), 7.18–7.25 (m, 5H, phenyl H-2, H-3, H-4, H-5, H-6), 7.26 (d, J = 8.5 Hz, 2H, 4-ethylphenyl H-3, H-5), 7.37 (d, J = 8.2 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.82–7.86 (m, 4H, 4-methanesulfonylphenyl H-3, H-5; 4-ethylphenyl H-2, H-6). Anal. ($C_{26}H_{22}O_4S$): C, H.

4-(4-Methanesulfonylphenyl)-3-phenyl-6-(4-trifluoromethylphenyl)-pyran-2-one (12n). The product was obtained as a yellow solid by oxidation of **11n** in the presence of aqueous Oxone® solution (0.19 g, 75.4%): mp 203–205 °C; IR (film): 1705 (C=O), 1315, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.05 (s, 3H, SO_2CH_3), 6.86 (s, 1H, pyranone H-5), 7.14–7.19 (m, 2H, phenyl H-2, H-6), 7.23–7.35 (m, 3H, phenyl H-3, H-4, H-5), 7.38 (d, J = 8.8 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.74 (d, J = 8.5 Hz, 2H, 4-trifluoromethylphenyl H-2, H-6), 7.77 (d, J = 8.8 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 8.01 (d, J = 8.5 Hz, 2H, 4-trifluoromethylphenyl H-3, H-5). Anal. ($C_{25}H_{17}F_3O_4S$): C, H.

6-(4-Methoxyphenyl)-4-(4-methanesulfonylphenyl)-3-phenylpyran-2-one (12o). The product was obtained as a yellow solid by oxidation of **11o** in the presence of aqueous Oxone® solution (0.19 g, 81.3%): mp 221–223 °C; IR (film): 1709 (C=O), 1311, 1151 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.04 (s, 3H, SO_2CH_3), 3.89 (s, 3H, OCH_3), 6.66 (s, 1H, pyranone H-5), 6.98 (d, J = 8.5 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.13–7.17 (m, 2H, phenyl H-2, H-6), 7.20–7.33 (m, phenyl H-3, H-4, H-5), 7.37 (d, J = 8.8 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.81–7.89 (m, 4H, 4-methanesulfonylphenyl H-3, H-5; 4-methoxyphenyl H-2, H-6). Anal. ($C_{25}H_{20}O_5S$): C, H.

6-(4-Ethoxyphenyl)-4-(4-methanesulfonylphenyl)-3-phenylpyran-2-one (12p). The product was obtained as a yellow solid by oxidation of **11p** in the presence of aqueous Oxone® solution (0.16 g, 70%): mp 181–183 °C; IR (film): 1703 (C=O), 1315, 1157 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.44 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 3.05 (s, 3H, SO_2CH_3), 4.08 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.66 (s, 1H, pyranone H-5), 6.95 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-3, H-5), 7.14–7.18 (m, 2H, phenyl H-2, H-6), 7.21–7.30 (m, 3H, H-3, H-4, H-5), 7.37 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.83–7.87 (m, 4H, 4-ethoxyphenyl H-2, H-6; 4-methanesulfonylphenyl H-3, H-5). Anal. ($C_{26}H_{22}O_5S$): C, H.

6-(4-Fluorophenyl)-4-(4-methanesulfonylphenyl)-3-phenylpyran-2-one (12q). The product was obtained as a yellow solid by oxidation of **11q** in the presence of aqueous Oxone® solution (0.17 g, 78%): mp 233–235 °C; IR (film): 1716 (C=O), 1312, 1143 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.05 (s, 3H, SO_2CH_3), 6.72 (s, 1H, pyranone H-5), 7.14–7.20 (m, 4H, 4-fluorophenyl H-3, H-5; phenyl H-2, H-6),

7.21–7.25 (m, 3H, phenyl H-3, H-4, H-5), 7.26 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.84 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5), 7.87 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6). Anal. ($C_{24}H_{17}FO_4S$): C, H.

3-(4-Fluorophenyl)-6-(4-methoxyphenyl)-4-(4-methanesulfonylphenyl)pyran-2-one (12r). The product was obtained as a yellow solid by oxidation of **11r** in the presence of aqueous Oxone® solution (0.20 g, 84%): mp 228–230 °C; IR (film): 1715 (C=O), 1315, 1155 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.05 (s, 3H, SO_2CH_3), 3.87 (s, 3H, OCH_3), 6.66 (s, 1H, pyranone H-5), 6.91–7.01 (m, 4H, 4-fluorophenyl H-3, H-5; 4-methoxyphenyl H-3, H-5), 7.11 (dd, J^3_{HH} = 8.4, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.37 (d, J = 8.2 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.83–7.89 (m, 4H, 4-methoxyphenyl H-2, H-6; methanesulfonylphenyl H-3, H-5). Anal. ($C_{25}H_{19}FO_5S$): C, H.

3-(4-Fluorophenyl)-6-(4-ethoxyphenyl)-4-(4-methanesulfonylphenyl)pyran-2-one (12s). The product was obtained as a yellow solid by oxidation of **11s** in the presence of aqueous Oxone® solution (0.23g, 92.5%): mp 210–212 °C; IR (film): 1698 (C=O), 1316, 1157 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.44 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 3.05 (s, 3H, SO_2CH_3), 4.07 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.65 (s, 1H, pyranone H-5), 6.90–7.00 (m, 4H, fluorophenyl H-3, H-5; 4-ethoxyphenyl H-3, H-5), 7.08 (d, J = 8.5 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.11 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.37 (d, J = 8.2 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.82–7.88 (m, 4H, 4-methanesulfonylphenyl H-3, H-5; 4-ethoxyphenyl H-2, H-6). Anal. ($C_{26}H_{21}FO_5S$): C, H.

General Procedure for the Synthesis of 6-(4-Methylsulfanylphenyl)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (12t) and 6-(4-Methylsulfanylphenyl)-3-(4-methanesulfonylphenyl)-4-(4-fluorophenyl)pyran-2-one (12u). To a stirred solution of the ethyl 4-methanesulfonylphenylacetate (**10d,** 1.55 mmol) in *t*-butanol (10 mL) at 30 °C was added potassium-*t*-butoxide (1.90 mmol), followed by a solution of **9g** or **9k** (1.58 mmol) in *t*-butanol (10 mL), and the reaction mixture was heated at 50–60 °C for 1–1.5 h under an argon atmosphere. The reaction mixture was cooled to 25 °C, washed with 1N HCl solution (10 mL), extracted with EtOAc (2 x 20 mL), the organic organic phase was separated, dried over Na_2SO_4, and the solvent was removed in vacuo to give a dark reddish oil which was purified by silica gel column chromatography using hexane–ethyl acetate (1:2, v/v or 1:3, v/v) as eluent to afford the respective title compound **12t** or **12u** (in 17 and 34% yield respectively). Some physical and spectroscopic data for **12t** and **12u** are listed below.

6-(4-Methylsulfanylphenyl)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (12t). The product was obtained as a yellow solid by condensation of **9g** with **10d** in the presence of potassium-*t*-butoxide (0.24 g, 34%): mp 231–233 °C; IR (film): 1709 (C=O), 1315, 1157 (SO_2) cm^{-1}; ^1H NMR

($CDCl_3$): δ 2.52 (s, 3H, SCH_3), 3.03 (s, 3H, SO_2CH_3), 6.80 (s, 1H, pyranone H-5), 7.12–7.15 (m, 2H, phenyl H-2, H-6), 7.25–7.34 (m, 5H, 4-methylsulfanylphenyl H-3, H-5; phenyl H-3, H-4, H-5), 7.40 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.78–7.99 (m, 4H, methanesulfonylphenyl H-3, H-5; 4-methylsulfanylphenyl H-2, H-6). Anal. ($C_{25}H_{20}O_4S_2$): C, H.

4-(4-Fluorophenyl)-6-(4-methylsulfanylphenyl)-3-(4-methanesulfonylphenyl)pyran-2-one (12u). The product was obtained as a yellow solid by condensation of **9k** with **10d** in the presence of potassium-*t*-butoxide (0.13 g, 17.6%): mp 216–218 °C; IR (film): 1703 (C=O), 1313, 1159 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.50 (s, 3H, SCH_3), 3.04 (s, 3H, SO_2CH_3), 6.79 (s, 1H, pyranone H-5), 6.95 (dd, J^3_{HH} = 8.5, J^3_{FH} = 8.5 Hz, 2H, 4-fluorophenyl H-3, H-5), 7.10 (dd, J^3_{HH} = 8.5, J^4_{FH} = 5.2 Hz, 2H, 4-fluorophenyl H-2, H-6), 7.26 (d, J = 8.5 Hz, 2H, 4-methysulfanylphenyl H-3, H-5), 7.38 (d, J = 8.8 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.81–7.92 (m, 4H, 4-methanesulfonylphenyl H-3, H-5; 4-methylsulfanylphenyl H-2, H-6). Anal. ($C_{25}H_{19}FO_4S_2$): C, H.

Procedure for the Synthesis of 1-(4-Nitrophenyl)-3-phenylprop-2-yn-1-one (9s). To a mixture of phenylacetylene (1.0 g, 9.8 mmol) and copper (I) iodide (0.09 g, 0.50 mmol), Et_3N (30 mL) was added under an argon atmosphere. 4-Nitrobenzoyl chloride (12.2 mmol) was then added slowly and the reaction mixture was stirred at 25 °C for 30 h at which time the reaction mixture was diluted with EtOAc (25 mL) and the mixture was filtered. The filtrate was evaporated under reduced pressure and the residue obtained was further purified by a silica gel column chromatography using hexane–ethyl acetate (1:3, v/v) as eluent to afford the title compound **9s** as a yellow solid in 24.6% yield: (0.61 g): mp 157–159 °C (lit. 162.5–163 °C[38]); IR (film): 2192 (C≡C), 1643 (C=O), 1535, 1348 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.45–7.57 (m, 4H, nitrophenyl H-3, H-5; phenyl H-2, H-6), 7.71 (d, J = 8.4 Hz, 2H, nitrophenyl H-2, H-6), 8.27–8.39 (m, 3H, 4-phenyl H-3, H-4, H-5).

3-(4-Methylsulfanylphenyl)-6-(4-nitrophenyl)-4-phenylpyran-2-one (11t). The product was obtained as a yellow solid by condensation of **9s** with **10b** in the presence of NaH (0.24 g, 34.4%): mp 182–184 °C; IR (film): 1716 (C=O), 1532, 1351 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.46 (s, 3H, SCH_3), 6.97 (s, 1H, pyranone H-5), 7.11 (d, J = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.15 (d, J = 8.5 Hz, 2H, methylsulfanylphenyl H-2, H-6), 7.18–7.21 (m, 2H, phenyl H-2, H-6), 7.29–7.35 (m, 3H, phenyl H-3, H-4, H-5), 8.06 (d, J = 8.8 Hz, 2H, nitrophenyl H-2, H-6), 8.33 (d, J = 8.8 Hz, 2H, nitrophenyl H-3, H-5). Anal. ($C_{24}H_{17}NO_4S$): C, H, N.

3-(4-Methanesulfonylphenyl)-6-(4-nitrophenyl)-4-phenylpyran-2-one (12v). The product was obtained as a yellow solid by the oxidation of **11t** in the presence of aqueous Oxone® solution (0.18 g, 76%): mp 238–240 °C; IR (film): 1696 (C=O), 1528, 1353 (NO_2), 1320, 1148 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.04 (s, 3H, SO_2CH_3), 7.02 (s, 1H, pyranone H-5), 7.13–7.15 (m, 2H, phenyl H-2, H-6), 7.29–7.35 (m, 3H, phenyl H-3, H-4, H-5), 7.41 (d, J = 8.5 Hz, 2H, 4-

methanesulfonylphenyl H-2, H-6), 7.82 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5), 8.08 (d, J = 8.8 Hz, 2H, nitrophenyl H-2, H-6), 8.35 (d, J = 8.8 Hz, 2H, nitrophenyl H-3, H-5). Anal. ($C_{24}H_{17}NO_6S$): C, H, N.

Synthesis of 6-(4-Aminophenyl)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (12w). To a stirred solution of **12v** (0.09 g, 0.22 mmol) in 95% ethanol (15 mL) was added palladium on activated carbon (4 mg). The reaction mixture was warmed to 50 °C with stirring, $NH_2NH_2.H_2O$ (0.04 mL, 0.82 mmol) was added drop wise, and the reaction mixture was refluxed at 75–78 °C for 1 h. After cooling to 25 °C, the reaction mixture was filtered, washed with water (10 mL), extracted with EtOAc (3 x 10 mL), the organic phase was separated, dried (Na_2SO_4), and the solvent was removed in vacuo to give a yellowish brown solid which was purified by recrystallization from ethanol (0.07 g, 76%): mp 252–254 °C; IR (film): 3429 (NH_2), 1670 (C=O), 1315, 1143 (SO_2) cm^{-1}; ^1H NMR (DMSO–d_6): δ 3.17 (s, 3H, SO_2CH_3), 5.96 (s, 2H, NH_2), 6.63 (d, J = 8.5 Hz, 2H, 4-aminophenyl H-3, H-5), 6.92 (s, 1H, pyranone H-5), 7.19–7.21 (m, 2H, phenyl H-2, H-6), 7.22–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.36 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.68–7.76 (m, 4H, 4-methanesulfonylphenyl H-3, H-5; 4-aminophenyl H-2, H-6). Anal. ($C_{24}H_{19}NO_4S$): C, H, N.

Procedure for the Synthesis of 6-(4-Hydroxyphenyl)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (12x). A mixture of **12e** (0.27 g, 0.63 mmol) and pyridinium hydrochloride (2.5 g, 21.7 mmol) was heated at 190–210 °C for 1–1.5 h. The reaction mixture was cooled, washed with water (20 mL) and extracted with EtOAc (3 x 20 mL), the organic phase was separated, and dried (Na_2SO_4). Removal of the solvent in vacuo gave a dark brown oil which was purified by silica gel column chromatography using hexane–ethyl acetate (1:3, v/v or) as eluent to afford **12x** (0.05 g, 20%) as a yellowish brown solid: mp 300–301 °C; IR (film): 3462 (OH), 1705 (C=O), 1320, 1148 (SO_2) cm^{-1}; ^1H NMR (DMSO–d_6): δ 3.20 (s, 3H, SO_2CH_3), 6.77 (s, 1H, pyranone H-5), 6.88 (d, J = 8.8 Hz, 2H, 4-hydroxyphenyl H-3, H-5), 7.21–7.26 (m, 2H, phenyl H-2, H-6), 7.29–7.33 (m, 3H, phenyl H-3, H-4, H-5), 7.39 (d, J = 8.4 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.76 (d, J = 8.8 Hz, 2H, 4-hydroxyphenyl H-2, H-6), 7.86 (d, J = 8.4 Hz, 2H, methanesulfonylphenyl H-3, H-5), 10.24 (broad s, 1H, O*H*). Anal. ($C_{24}H_{18}O_5S$): C, H.

Preparation of 1-Ethynyl-4-methanesulfonylbenzene. To a stirred solution of 1-ethynyl-4-methylsulfanylbenzene (3.0 g, 20 mmol) in 1,4-dioxane (25 ml) at 0 °C, 50% w/v aqueous solution of Oxone® (60 mmol) was added drop wise, and the reaction mixture was stirred at 25 °C for 4–5 h. The reaction mixture was diluted with water (15 mL), extracted with EtOAc (2 x 30 mL), and washed successively with brine solution and then water (15 mL each), the organic organic phase was separated, dried (Na_2SO_4) and the solvent was removed in vacuo to give a crude oil which was purified by silica gel column chromatography using hexane–ethyl acetate (1:2, v/v or 1:3, v/v) as eluent to afford the title compound in 74% yield as a brownish oil (2.2 g). IR (film): 2119

(C≡C), 1351, 1158 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.06 (s, 3H, SO_2CH_3), 3.30 (s, 1H, C≡C*H*), 7.66 (d, *J* = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.90 (d, *J* = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5).

3-(4-Methanesulfonylphenyl)-1-(4-methylsulfanylphenyl)prop-2-yn-1-ol (8s). This product was obtained as a brownish oil by the reaction of 1-ethynyl-4-methanesulfonylbenzene with 4-methylthiobenzaldehyde in the presence of *n*-BuLi (2.5 g, 77.3%): IR (film): 3410 (OH), 2267 (C≡C), 1306, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.20 (d, *J* = 6.1 Hz, 1H,CHO*H*), 2.50 (s, 3H, SCH_3), 3.06 (s, 3H, SO_2CH_3), 5.67 (d, *J* = 6.1 Hz, 1H,C*H*OH), 7.28 (d, *J* = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.50 (d, *J* = 8.2 Hz, 2H, 4-methylsulfanylphenyl H-2, H-6), 7.62 (d, *J* = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.88 (d, *J* = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5). Anal. ($C_{17}H_{16}O_3S_2$): C, H.

3-(4-Methanesulfonylphenyl)-1-(4-methylsulfanylphenyl)prop-2-yn-1-one (9t). This product was obtained as a brown oil by the oxidation of **8s** in the presence of MnO_2 (1 g, 67%): IR (film): 2200 (C≡C), 1635 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.51 (s, 3H, SCH_3), 3.06 (s, 3H, SO_2CH_3), 7.31 (d, *J* = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.84 (d, *J* = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.92 (d, *J* = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5). 8.09 (d, *J* = 8.5 Hz, 2H, methylsulfanylphenyl H-3, H-5). Anal. ($C_{17}H_{14}O_3S_2$): C, H.

6-(4-Methylsulfanylphenyl)-4-(4-methanesulfonylphenyl)-3-phenylpyran-2-one (12y). The product was obtained as a yellow solid by condensation of **9t** with **10a** in the presence of potassium-*t*-butoxide (0.32 g, 45.8%): mp 223–225 °C; IR (film): 1716 (C=O), 1320, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.51 (s, 3H, SCH_3), 3.05 (s, 3H, SO_2CH_3), 6.73 (s, 1H, pyranone H-5), 7.13–7.17 (m, 2H, phenyl H-2, H-6), 7.20–7.25 (m, phenyl H-3, H-4, H-5), 7.26 (d, *J* = 8.5 Hz, 2H, 4-methylsulfanylphenyl H-3, H-5), 7.37 (d, *J* = 8.2 Hz, 2H, methanesulfonylphenyl H-2, H-6), 7.80–7.86 (m, 4H, 4-methanesulfonylphenyl H-3, H-5; 4-methylsulfanylphenyl H-2, H-6). Anal. ($C_{25}H_{20}O_4S_2$): C, H.

1-Phenyl-3-pyridin-3-yl-prop-2-yn-1-ol (8t). The product was obtained as a dark brown semisolid by reaction of pyridin-3-ylacetylene with benzaldehyde in the presence of *n*-BuLi (0.39 g, 40%): IR (film): 3160 (OH), 2260 (C≡C), cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.89 (broad s, CHO*H*), 5.73 (s, 1H,C*H*OH), 7.24 (dd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 4.7 Hz, 1H, pyridinyl H-5), 7.34–7.46 (m, 3H, phenyl H-3, H-4, H-5), 7.61–7.63 (m, 2H, phenyl H-2, H-6), 7.75 (dd, $J_{4,5}$ = 8.0 Hz, $J_{4,6}$ = 2.2 Hz, 1H, pyridinyl H-4), 8.52 (dd, $J_{5,6}$ = 4.7 Hz, $J_{4,6}$ = 2.2 Hz, 1H, pyridinyl H-6), 8.75 (d, $J_{2,4}$ = 2.2 Hz, 1H, pyridinyl H-2). Anal. ($C_{14}H_{11}NO$): C, H, N.

1-(4-Methoxyphenyl)-3-pyridin-3-yl-prop-2-yn-1-ol (8u). The product was obtained as a soild by reaction of pyridin-3-ylacetylene with 4-methoxybenzaldehyde in the presence of *n*-BuLi (1.22 g, 52.3%): mp 86–88 °C; IR (film): 3360 (OH), 2267 (C≡C), cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.83 (broad s, 1H, CHO*H*), 3.83 (s, 3H, OCH_3), 5.67 (s, 1H,C*H*OH), 6.97 (d, *J* = 8.2 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.25 (dd, $J_{4,5}$

= 8.0 Hz, $J_{5,6}$ = 4.7 Hz, H, pyridinyl H-5), 7.52 (d, J = 8.2 Hz, 2H, 4-methoxyphenyl H-2, H-6), 7.74 (dd, $J_{4,5}$ = 8.0 Hz, $J_{4,6}$ = 2.4 Hz, 1H, pyridinyl H-4), 8.52 (dd, $J_{5,6}$ = 4.7 Hz, $J_{4,6}$ = 2.4 Hz, 1H, pyridinyl H-6), 8.73 (d, $J_{2,4}$ = 2.4 Hz, 1H, pyridinyl H-2). Anal. ($C_{15}H_{13}NO_2$): C, H, N.

1-Phenyl-3-pyridin-3-yl-prop-2-yn-1-one (9u). The product was obtained as solid by oxidation of **8t** in the presence of MnO_2 (0.56 g, 60.5%): mp 66–68 °C; IR (film): 2200 (C≡C), 1635 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.35 (dd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 4.7 Hz, 1H, pyridinyl H-5), 7.40–7.70 (m, 3H, phenyl H-3, H-4, H-5), 7.95 (dd, $J_{4,5}$ = 8.0 Hz, $J_{4,6}$ = 2.2 Hz, 1H, pyridinyl H-4), 8.20–8.24 (m, 2H, phenyl H-2, H-6), 8.70 (dd, $J_{5,6}$ = 4.7 Hz, $J_{4,6}$ = 2.2 Hz, 1H, pyridinyl H-6), 8.92 (d, $J_{2,4}$ = 2.2 Hz, 1H, pyridinyl H-2). Anal. ($C_{14}H_9NO$): C, H, N.

1-(4-Methoxyphenyl)-3-pyridin-3-yl-prop-2-yn-1-one (9v). The product was obtained as a white solid by oxidation of **8u** in the presence of MnO_2 (0.61 g, 57.3%): mp 126–128 °C; IR (film): 2206 (C≡C), 1642 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.92 (s, 3H, OCH_3), 6.99 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.36 (dd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 4.7 Hz, 1H, pyridinyl H-5), 7.95 (dd, $J_{4,5}$ = 8.0 Hz, $J_{4,6}$ = 2.4 Hz, 1H, pyridinyl H-4), 8.18 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-2, H-6), 8.68 (dd, $J_{5,6}$ = 4.7 Hz, $J_{4,6}$ = 2.4 Hz, 1H, pyridinyl H-6), 8.90 (d, $J_{2,4}$ = 2.4 Hz, 1H, pyridinyl H-2). Anal. ($C_{15}H_{11}NO_2$): C, H, N.

General Procedure for the Synthesis of 3-(4-Methanesulfonylphenyl)-6-phenyl-4-pyridin-3-yl-pyran-2-one (13a) and 6-(4-Methoxyphenyl)-3-(4-methanesulfonylphenyl)-4-pyridin-3-yl-pyran-2-one (13b). To a stirred solution of ethyl 4-methanesulfonylphenylacetate (**10d**, 0.24 g, 1.0 mmol) in CH_2Cl_2 (10 mL) was added NaH (95% dry powder, 1.1 mmol). A solution of the 1,3-diarylprop-2-yn-1-one (**9u** or **9v**, 1 mmol) in CH_2Cl_2 (10 mL) was added slowly, and the reaction mixture was stirred at 25 °C for 1 h. Removal of the solvent in vacuo gave a residue that was purified by silica gel column chromatography using hexane–ethyl acetate (1:3, v/v) as eluent to afford the respective title product **13a** or **13b** in 24–46% yield. Some physical and spectroscopic data for **13a** or **13b** are listed below.

3-(4-Methanesulfonylphenyl)-6-phenyl-4-pyridin-3-yl-pyran-2-one (13a). The product was obtained as a yellow solid by the condensation of **9u** with **10d** in the presence of NaH (0.09 g, 24%): mp 269–271 °C; IR (film): 1709 (C=O), 1327, 1162 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.05 (s, 3H, SO_2CH_3), 6.86 (s, 1H, pyranone H-5), 7.21 (dd, $J_{4,5}$ = 7.6 Hz, $J_{5,6}$ = 4.9 Hz, 1H, pyridinyl H-5), 7.39 (dd, $J_{4,5}$ = 8.0 Hz, $J_{4,6}$ = 2.4 Hz, 1H, pyridinyl H-4), 7.41 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.50–7.56 (m, 3H, phenyl H-3, H-4, H-5), 7.83 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.92 (dd, $J_{5,6}$ = 4.9 Hz, $J_{4,6}$ = 2.4 Hz, 1H, pyridinyl H-6), 8.53 (d, $J_{2,4}$ = 2.4 Hz, 1H, pyridinyl H-2). Anal. ($C_{23}H_{17}NO_4S$): C, H, N.

6-(4-Methoxyphenyl)-3-(4-methanesulfonylphenyl)-4-pyridin-3-yl-pyran-2-one (13b). The product was obtained as a yellow solid by the condensation of **9v** with **10d** in the presence of NaH (0.2 g, 46%): mp 268–

270 °C; IR (film): 1720 (C=O), 1320, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.04 (s, 3H, SO_2CH_3), 3.92 (s, 3H OCH_3), 6.74 (s, 1H, pyranone H-5), 7.00 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.19 (dd, $J_{4,5}$ = 8.0 Hz, $J_{5,6}$ = 4.9 Hz, 1H, pyridinyl H-5), 7.36 (dd, $J_{4,5}$ = 8.0 Hz, $J_{4,6}$ = 2.4 Hz, 1H, pyridinyl H-4), 7.40 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.81–7.90 (m, 4H, 4-methanesulfonylphenyl H-3, H-5; 4-methoxyphenyl H-2, H-6), 8.51 (dd, $J_{5,6}$ = 4.9 Hz, $J_{4,6}$ = 2.4 Hz, 1H, pyridinyl H-6), 8.57 (d, $J_{2,4}$ = 2.4 Hz, 1H, pyridinyl H-2). Anal. ($C_{24}H_{19}NO_5S$): C, H, N.

Preparation of 3-(4-Methanesulfonylphenyl)-1-phenylprop-2-yn-1-one (9w) and 3-(4-Methanesulfonylphenyl)-1-(4-methoxyphenyl)prop-2-yn-1-one (9x). To a stirred solution of the 1,3-diarylprop-2-yn-1-one (**9l** or **9p**, 0.54 mmol) in 1,4-dioxane (10 mL) at 0 °C, a 50% w/v aqueous solution of Oxone® (1.62 mmol) was added drop wise, and the reaction mixture was stirred at 25 °C for 4–5 h. The reaction mixture was diluted with water (10 mL), extracted with EtOAc (2 x 20 mL), washed successively with brine solution and water (10 mL each), the organic organic phase was separated, and dried (Na_2SO_4). Removal of the solvent in vacuo gave a crude oil which was purified by silica gel column chromatography using hexane–ethyl acetate (1:3, v/v) as eluent to afford the respective title compound **9w** or **9x** in 60–85% yield. Some physical and spectroscopic data for **9w** and **9x** are listed below.

3-(4-Methanesulfonylphenyl)-1-phenylprop-2-yn-1-one (9w). The product was obtained as a pale yellow solid by oxidation of **9l** in the presence of aqueous Oxone® solution (0.11 g, 74%): mp 111–113 °C; IR (film): 2206 (C≡C), 1605 (C=O), 1320, 1139 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.06 (s, 3H, SO_2CH_3), 7.53–7.71 (m, phenyl H-3, H-4, H-5), 7.86 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 8.01 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 8.20–8.23 (m, 2H, phenyl H-2, H-6). Anal. ($C_{16}H_{12}O_3S$): C, H.

3-(4-Methanesulfonylphenyl)-1-(4-methoxyphenyl)prop-2-yn-1-one (9x). The product was obtained as a pale yellow solid by oxidation of **9p** in the presence of aqueous Oxone® solution (0.11 g, 66.3%): mp 157–159 °C; IR (film): 2206 (C≡C), 1604 (C=O), 1320, 1140 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.10 (s, 3H, SO_2CH_3), 3.90 (s, 3H, OCH_3), 7.00 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.84 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 8.01 (d, J = 8.5 Hz, 2H, methanesulfonylphenyl H-3, H-5), 8.17 (d, J = 8.8 Hz 4-methoxyphenyl H-2, H-6). Anal. ($C_{17}H_{14}O_4S$): C, H.

Preparation of Pyridin-3-yl or Pyridin-4-yl-pyran-2-ones (13c–f). To a stirred solution of either the pyridin-3-yl or pyridin-4-ylacetic acid ester (1.0 mmol) in CH_2Cl_2 (10 mL) was added NaH (95% dry powder, 1.1 mmol). A solution of the respective 1,3-diarylprop-2-yn-1-one (**9w–x**, 1.0 mmol) in CH_2Cl_2 (10 mL) was added slowly, and the reaction mixture was allowed to stir at 25 °C for 1 h. Removal of the solvent in vacuo gave a residue that was purified by silica gel column chromatography using hexane–ethyl acetate (1:3, v/v) as

eluent to afford the respective title product **13c–f** in 24–46% yield. Some physical and spectroscopic data for **13c–f** are listed below.

4-(4-Methanesulfonylphenyl)-6-phenyl-3-pyridin-3-yl-pyran-2-one (13c). The product was obtained as yellow solid by the condensation of **9w** with the pyridin-3-ylacetic acid ester in presence of NaH (0.15 g, 38.2%): mp 235–237 °C; IR (film): 1690 (C=O), 1313, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.06 (s, 3H, SO_2CH_3), 6.82 (s, 1H, pyranone H-5), 7.24 (dd, $J_{4,5}$ = 7.0 Hz, $J_{5,6}$ = 4.9 Hz, 1H, pyridinyl H-5), 7.29 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.41–7.50 (m, 3H, phenyl H-3, H-4, H-5), 7.71 (dd, $J_{4,5}$ = 7.0 Hz, $J_{4,6}$ = 2.4 Hz, 1H, pyridinyl H-4), 7.88 (d, J = 8.5 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.92–7.94 (m, 2H, phenyl H-2, H-6), 8.28 (dd, $J_{5,6}$ = 4.9 Hz, $J_{4,6}$ = 2.4 Hz, 1H, pyridinyl H-6), 8.48 (d, $J_{2,4}$ = 2.4 Hz, 1H, pyridinyl H-2). Anal. ($C_{23}H_{17}NO_4S$): C, H, N.

6-(4-Methoxyphenyl)-4-(4-methanesulfonylphenyl)-3-pyridin-3-yl-pyran-2-one (13d). The product was obtained as a yellow solid by the condensation of **9x** with the pyridin-3-ylacetic acid ester in the presence of NaH (0.18 g, 42.3%): mp 242–244 °C; IR (film): 1703 (C=O), 1306, 1165 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.05 (s, 3H, SO_2CH_3), 3.89 (s, 3H OCH_3), 6.70 (s, 1H, pyranone H-5), 7.00 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.25 (dd, $J_{4,5}$ = 7.9 Hz, $J_{5,6}$ = 5.2 Hz, 1H, pyridinyl H-5), 7.38 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.75 (dd, $J_{4,5}$ = 7.9 Hz, $J_{4,6}$ = 1.8 Hz, 1H, pyridinyl H-4), 7.86–7.91 (m, 4H, 4-methanesulfonylphenyl H-3, H-5; 4-methoxyphenyl H-2, H-6), 8.27 (dd, $J_{5,6}$ = 5.2 Hz, $J_{4,6}$ = 1.8 Hz, 1H, pyridinyl H-6), 8.49 (d, $J_{2,4}$ = 1.8 Hz, 1H, pyridinyl H-2). Anal. ($C_{24}H_{19}NO_5S$): C, H, N.

4-(4-Methanesulfonylphenyl)-6-phenyl-3-pyridin-4-yl-pyran-2-one (13e). The product was obtained as a yellow solid by the condensation of **9x** with the pyridin-4-ylacetic acid ester in the presence of NaH (0.09 g, 22.4%): mp 255–257 °C; IR (film): 1712 (C=O), 1320, 1152 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.07 (s, 3H, SO_2CH_3), 6.80 (s, 1H, pyranone H-5), 7.11 (d, $J_{2,3}$ = $J_{5,6}$ = 5.5 Hz, 2H, pyridinyl H-3, H-5), 7.39 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.44–7.53 (m, 3H, phenyl H-3, H-4, H-5), 7.89 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-3, H-5), 7.92–7.95 (m, 2H, phenyl H-2, H-6), 8.50 (d, $J_{2,3}$ = $J_{5,6}$ = 5.5 Hz, 2H, pyridinyl H-2, H-6). Anal. ($C_{23}H_{17}NO_4S$): C, H, N.

6-(4-Methoxyphenyl)-4-(4-methanesulfonylphenyl)-3-pyridin-4-yl-pyran-2-one (13f). The product was obtained as a yellow solid by the condensation of **9x** with the pyridin-4-ylacetic acid ester in the presence of NaH (0.17 g, 41.3%): mp 240–242 °C; IR (film): 1716 (C=O), 1320, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.06 (s, 3H, SO_2CH_3), 3.90 (s, 3H OCH_3), 6.69 (s, 1H, pyranone H-5), 6.98 (d, J = 8.5 Hz, 2H, 4-methoxyphenyl H-3, H-5), 7.13 (d, $J_{2,3}$ = $J_{5,6}$ = 5.5 Hz, 2H, pyridinyl H-3, H-5), 7.38 (d, J = 8.2 Hz, 2H, 4-methanesulfonylphenyl H-2, H-6), 7.85–7.93 (m, 4H, 4-methanesulfonylphenyl H-3, H-5; 4-methoxyphenyl H-2, H-6), 8.49 (d, $J_{2,3}$ = $J_{5,6}$ = 5.5 Hz, 2H, pyridinyl H-2, H-6). Anal. ($C_{24}H_{19}NO_5S$): C, H, N.

Preparation of 4-Azidophenylacetic acid (15). To an ice cold solution of 4-aminophenylacetic acid (**14**, 2.0 g, 13.2 mmol) in conc. HCl (20 mL) was added an aqueous solution of $NaNO_2$ (13.3 mmol, 0.92 g in 70 mL water) slowly with stirring. After 15 min, an aqueous solution of NaN_3 (132 mmol, 8.6 g in 200 mL of water) was added at 0 °C over a period of 15 min after which the reaction mixture was stirred at 25 °C for 15–20 min. The reaction mixture was extracted with EtOAc (3 x 20 mL), the organic phase was separated, dried over Na_2SO_4, and the solvent was removed in vacuo to give brown crystals of **15**. (2.2 g, 94%): mp 86–88 °C; IR (film): 2119 (N_3), 1710 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.62 (s, 2H, CH_2CO), 6.98 (d, J = 8.8 Hz, 2H, 4-azidophenyl H-3, H-5), 7.29 (d, J = 8.8 Hz, 2H, azidophenyl H-2, H-6). Anal. ($C_8H_7N_3O_2$): C, H, N.

Preparation of Ethyl-4-azidophenylacetate (16). To a stirred solution of 4-azidophenylacetic acid (**15**, 1.0 g, 5.6 mmol) in conc. H_2SO_4 (1.1 mL) was added ethanol (5.2 mL of 95%) and the reaction mixture was refluxed at 70–75 °C for 3–4 h. The reaction mixture was diluted with EtOAc (15 mL) and $NaHCO_3$ (1 g), was added, filtered and the organic fraction was dried (Na_2SO_4). The EtOAc fraction was evaporated to give a brownish oil (1 g, 87%): IR (film): 2131 (N_3), 1739 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.23 (t, J = 7.0 Hz, 3H, $COCH_2CH_3$), 3.59 (s, 2H, CH_2CO), 4.12 (q, J = 7.0 Hz, 2H, $COCH_2CH_3$), 6.98 (d, J = 8.8 Hz, 2H, 4-azidophenyl H-3, H-5), 7.24 (d, J = 8.8 Hz, 2H, azidophenyl H-2, H-6). Anal. ($C_{10}H_{11}N_3O_2$): C, H, N.

General Procedure for the Synthesis of 3-(4-Azidophenyl)-6-(4-methoxy, ethoxy, or methylsulfanylphenyl)-4-phenylpyran-2-ones (17a–c). To a stirred solution of **16** (0.22 g, 1.7 mmol) in CH_2Cl_2 (10 mL) was added NaH (95% dry powder, 1.9 mmol). The 1,3-diarylprop-2-yn-1-one (**9e–g**, 1.7 mmol) in CH_2Cl_2 (10 mL) was added slowly, and the reaction mixture was allowed to stir at 25 °C for 1 h. Removal of the solvent in vacuo gave a residue that was purified by silica gel column chromatography using hexane–ethyl acetate (3:1, v/v) as eluent to afford the respective product **17a–c** in 28–33% yield. Some physical and spectroscopic data for **17a–c** are listed below.

3-(4-Azidophenyl)-6-(4-methoxyphenyl)-4-phenylpyran-2-one (17a). The product was obtained as orange crystals by the condensation of **9e** with **16** in presence of NaH (0.20 g, 30.7%): mp 184–186 °C; IR (film): 2112, 2105 (N_3), 1716 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.89 (s, 3H, OCH_3), 6.73 (s, 1H, pyranone H-5), 6.88 (d, J = 8.5 Hz, 2H, 4-azidophenyl H-3, H-5), 6.97 (d, J = 8.8 Hz, 2H, methoxyphenyl H-3, H-5), 7.17–7.21 (m, 4H, 4-azidophenyl H-2, H-6; phenyl H-2, H-6), 7.26–7.31 (m, 3H, phenyl H-3, H-4, H-5), 7.85 (d, J = 8.8 Hz, 2H, 4-methoxyphenyl H-2, H-6); ^{13}C NMR ($CDCl_3$): δ 55.5 (OCH_3), 103.5 (pyranone C-5), 114.4 (4-methoxyphenyl C-3, C-5), 118.6 (4-azidophenyl H-3, H-5), 120.8 (pyranone C-3), 123.8 (4-methoxyphenyl C-1), 127.3 (phenyl C-3, C-5), 128.5, 128.6 and 128.7 (phenyl C-4, 4-methoxyphenyl C-2, C-6; 4-azidophenyl C-2, C-6), 130.7 (4-azidophenyl C-4), 132.4 (4-phenyl C-2, C-6), 137.9 (phenyl C-1), 139.1 (4-azidophenyl C-1), 153.2 (pyranone C-4), 158.5 (4-methoxyphenyl C-4), 161.7

(pyranone C-6), 162.6 (pyranone C-2). Anal. ($C_{24}H_{17}N_3O_3$): C, H, N.

3-(4-Azidophenyl)-6-(4-ethoxyphenyl)-4-phenylpyran-2-one (17b). The product was obtained as a orange solid by the condensation of **9f** with **16** in the presence of NaH (0.24 g, 35.6%): mp 169–171 °C; IR (film): 2119, 2109 (N_3), 1709 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.35 (t, J = 7.0 Hz, 3H, OCH_2CH_3), 3.98 (q, J = 7.0 Hz, 2H, OCH_2CH_3), 6.64 (s, 1H, pyranone H-5), 6.75 (d, J = 8.8 Hz, 2H, ethoxyphenyl H-3, H-5), 6.88 (d, J = 8.5 Hz, 2H, 4-azidophenyl H-3, H-5), 7.06–7.12 (m, 4H, 4-azidophenyl H-2, H-6; phenyl H-2, H-6), 7.14–7.24 (m, 3H, phenyl H-3, H-4, H-5), 7.74 (d, J = 8.8 Hz, 2H, 4-ethoxyphenyl H-2, H-6); ^{13}C NMR ($CDCl_3$): δ 14.7 (OCH_2CH_3), 63.7 (OCH_2CH_3), 103.4 (pyranone C-5), 114.8 (4-ethoxyphenyl C-3, C-5), 118.6 (4-azidophenyl H-3, H-5), 120.7 (pyranone C-3), 123.6 (4-ethoxyphenyl C-1), 127.3 (phenyl C-3, C-5), 128.4, 128.6 and 128.7 (phenyl C-4, 4-ethoxyphenyl C-2, C-6; 4-azidophenyl C-2, C-6), 130.7 (4-azidophenyl C-4), 132.4 (4-phenyl C-2, C-6), 137.9 (phenyl C-1), 139.1 (4-azidophenyl C-1), 153.2 (pyranone C-4), 158.6 (4-ethoxyphenyl C-4), 161.2 (pyranone C-6), 162.6 (pyranone C-2). Anal. ($C_{25}H_{19}N_3O_3$): C, H, N.

3-(4-Azidophenyl)-6-(4-methylsulfanylphenyl)-4-phenylpyran-2-one (17c). The product was obtained as a pale yellow solid by the condensation of **9g** with **16** in the presence of NaH (0.23 g, 33.6%): mp 170–172 °C; IR (film): 2112, 2106 (N_3), 1709 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.55 (s, 3H, SCH_3), 6.79 (s, 1H, pyranone H-5), 6.88 (d, J = 8.5 Hz, 2H, 4-azidophenyl H-3, H-5), 7.15–7.20 (m, 4H, 4-azidophenyl H-2, H-6; phenyl H-2, H-6), 7.21–7.32 (m, 5H, 4-methylsulfanylphenyl H-3, H-5; phenyl H-3, H-4, H-5), 7.80 (d, J = 8.8 Hz, 2H, 4-methylsulfanylphenyl H-2, H-6); ^{13}C NMR ($CDCl_3$): δ 15.1 (SCH_3), 104.2 (pyranone C-5), 118.6 (4-azidophenyl C-3, C-5), 121.6 (pyranone C-3), 125.8 (4-methylsulfanylphenyl C-3, C-5), 127.5 (4-methylsulfanylphenyl C-1), 127.3 (phenyl C-3, C-5), 128.5, 128.5 and 128.8 (phenyl C-4, 4-methylsulfanylphenyl C-2, C-6; 4-azidophenyl C-2, C-6), 130.5 (4-azidophenyl C-4), 132.4 (4-phenyl C-2, C-6), 137.7 (phenyl C-1), 139.2 (4-azidophenyl C-1), 142.8 (4-methylsulfanylphenyl C-4), 152.9 (pyranone C-4), 158.1 (pyranone C-6), 162.5 (pyranone C-2). Anal. ($C_{24}H_{17}N_3O_2S$): C, H, N.

Cyclooxygenase Inhibition Studies. The ability of the test compounds **12, 13** and **17** to inhibit ovine COX-1 and COX-2 ((IC_{50} values, μM) was determined using an enzyme immuno assay (EIA) kit (catalog number 560101, Cayman Chemical, Ann Arbor, MI, U.S.A.) according to the manufacturer's instructions. Cyclooxygenase catalyzes the first step in the biosynthesis of arachidonic acid (AA) to PGH_2. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride, is measured by enzyme immunoassay (ACE™ competitive EIA). Stock solutions of test compounds were dissolved in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (960 μl, 0.1 M Tris-HCl pH 8.0 containing 5 mM EDTA and 2 mM phenol) with either COX-1 or COX-2 (10 μl) enzyme in the presence of heme (10 μl) were added 10 μl of various concentrations of

test drug solutions (0.001, 0.1, 1, 10, 50 and 100 μM in a final volume of 1 ml). These solutions were incubated for a period of 5 min at 37 °C after which 10 μl of AA (100 μM) solution were added and the COX reaction was stopped by the addition of 50 μl of 1 M HCl after 2 min. $PGF_{2\alpha}$, produced from PGH_2 by reduction with stannous chloride was measured by enzyme immunoassay. This assay is based on the competition between PGs and a PG-acetylcholinesterase conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGs in the wells since the concentration of PG tracer is held constant while the concentration of PGs varies. This antibody-PG complex binds to a mouse anti-rabbit monoclonal antibody that had been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's reagent, which contains the substrate to acetylcholine esterase, is added to the well. The product of this enzymatic reaction produces a distinct yellow color that absorbs at 405 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of PGs present in the well during the incubation: Absorbance α [Bound PG Tracer] α 1/PGs. Percent inhibition was calculated by the comparison of compound-treated to various control incubations. The concentration of the test compound causing 50% inhibition (IC_{50}, μM) was calculated from the concentration-inhibition response curve (duplicate determinations).

Antiinflammatory Assay. The test compounds were evaluated using the in vivo rat carrageenan-induced foot paw edema model reported previously.[39,40]

Analgesic Assay. Analgesic activity was determined using the 4% sodium chloride-induced writhing (abdominal constriction) assay as described previously.[41]

Molecular Modeling (Docking) Studies. Docking experiments were performed using Insight II software Version 2000.1 (Accelrys Inc.) running on a Silicon Graphics Octane 2 R14000A workstation. The coordinates for the X-ray crystal structure of the enzyme COX-2 were obtained from the RCSB Protein Data Bank and hydrogens were added. The ligand molecules were constructed using the Builder module and energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The docking experiment on COX-2 was carried out by superimposing the energy minimized ligand on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. The resulting ligand–enzyme complex was subjected to docking using the Affinity command in the Docking module of Insight II after defining subsets of the enzyme such that residues within 10 Å of the ligand were allowed to relax, while the remainder of the enzyme residues were fixed. The consistent valence force field (CVFF) or extensible systematic force field (ESFF) was employed for all docking purposes. The ligand–enzyme assembly was then subjected to a molecular dynamics (MD) simulation using the Discover module Version 2.98 at a constant temperature of 300 K with a 200 step equilibration for over 5000 iterations and a time step of 1

fs using a distance dependent dielectric constant 4r. The optimal binding orientation of the ligand–enzyme assembly obtained after docking was further minimized for 1000 iterations using the conjugate gradient method until a convergence of 0.001 kcal/mol Å was reached after which $E_{intermolecular}$ (kcal/mol) of the ligand–enzyme assembly was evaluated.

Supporting Information on Elemental Analysis Data is Available in Appendix 4.1.

5.6.0.0. References

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Appendix 4.1:

Elemental Analysis for Compounds **8, 9, 11, 12, 13** and **17**.

Cmpd	Empirical Formula	Calculated			Found		
		C	H	N	C	H	N
8c	$C_{17}H_{16}O$	86.40	6.82	–	86.02	6.95	–
8f	$C_{17}H_{16}O_2$	80.93	6.39	–	80.62	6.03	–
8g	$C_{16}H_{14}OS$	75.55	5.55	–	75.72	5.18	–
8i	$C_{16}H_{13}FO_2$	74.99	5.11	–	74.63	5.32	–
8j	$C_{17}H_{15}FO_2$	75.54	5.59	–	75.21	5.86	–
8k	$C_{16}H_{13}FOS$	70.56	4.81	–	70.42	4.66	–
8l	$C_{16}H_{14}OS$	75.55	5.55	–	75.26	5.21	–
8m	$C_{17}H_{16}OS$	76.08	6.01	–	75.82	5.95	–
8n	$C_{18}H_{18}OS$	76.56	6.42	–	76.23	6.66	–
8o	$C_{17}H_{13}F_3OS$	63.34	4.06	–	63.54	4.36	–
8p	$C_{17}H_{16}O_2S$	71.80	5.67	–	71.91	5.29	–
8q	$C_{18}H_{18}O_2S$	72.45	6.28	–	72.09	6.08	–
8r	$C_{16}H_{13}FOS.1/4H_2O$	69.48	4.88	–	69.85	4.81	–
8s	$C_{17}H_{16}O_3S_2$	61.42	4.85	–	61.22	4.82	–
8t	$C_{14}H_{11}NO$	80.36	5.30	6.69	80.59	5.66	6.53
8u	$C_{15}H_{13}NO_2$	75.30	5.48	5.85	75.29	5.52	5.82
9c	$C_{17}H_{14}O$	87.15	6.02	–	86.82	6.05	–
9d	$C_{16}H_9F_3O$	70.08	3.31	–	69.83	3.14	–
9g	$C_{16}H_{12}OS$	76.16	4.79	–	76.09	4.57	–

9i	$C_{16}H_{11}FO_2$	75.58	4.36	–	75.57	4.33	–
9j	$C_{17}H_{13}FO_2$	76.11	4.88	–	76.23	4.62	–
9k	$C_{16}H_{11}FOS$	71.09	4.10	–	71.34	4.33	–
9l	$C_{16}H_{12}OS$	76.16	4.79	–	76.55	4.46	–
9m	$C_{17}H_{14}OS$	76.66	5.30	–	76.85	5.21	–
9n	$C_{18}H_{16}OS.1/2\ H_2O$	74.63	5.87	–	75.16	6.03	–
9o	$C_{17}H_{11}F_3OS$	63.74	3.46	–	63.42	3.74	–
9p	$C_{17}H_{14}O_2S$	72.31	5.00	–	72.58	4.96	–
9q	$C_{18}H_{16}O_2S$	72.94	5.44	–	72.66	5.27	–
9r	$C_{16}H_{11}FOS$	71.09	4.10	–	71.39	4.14	–
9t	$C_{17}H_{14}O_3S_2$	61.79	4.27	–	61.41	4.52	–
9u	$C_{14}H_9NO$	81.14	4.38	6.76	81.44	4.66	6.68
9v	$C_{15}H_{15}NO_2$	75.94	4.67	5.90	76.05	4.54	5.93
9w	$C_{16}H_{12}O_3S$	67.59	4.25	–	67.22	4.50	–
9x	$C_{17}H_{14}O_4S$	64.95	4.49	–	64.58	4.38	–
11a	$C_{24}H_{18}O_2S.1/4\ H_2O$	76.80	4.93	–	76.94	4.78	–
11b	$C_{25}H_{20}O_2S$	78.09	5.24	–	78.41	5.14	–
11c	$C_{26}H_{22}O_2S.3/4H_2O$	75.72	5.70	–	75.98	5.38	–
11d	$C_{25}H_{17}F_3O_2S.1/4\ H_2O$	67.77	3.89	–	67.61	3.90	–
11e	$C_{25}H_{20}O_3S$	75.34	5.35	–	75.21	4.97	–
11f	$C_{26}H_{22}O_3S$	75.34	5.35	–	75.46	5.62	–
11g	$C_{24}H_{17}FO_2S$	74.21	4.41	–	74.42	4.21	–

11h	$C_{25}H_{19}FO_3S$	71.75	4.58	–	71.51	4.47	–
11i	$C_{26}H_{21}FO_3S$	78.09	5.24	–	78.33	5.61	–
11j	$C_{24}H_{18}O_2S$	77.81	4.90	–	77.53	4.80	–
11k	$C_{24}H_{18}O_2S$	77.81	4.90	–	77.45	4.77	–
11l	$C_{25}H_{20}O_2S$	78.09	5.24	–	78.25	5.14	–
11m	$C_{26}H_{22}O_2S.1/4\ H_2O$	77.41	5.58	–	77.58	5.38	–
11n	$C_{25}H_{17}F_3O_2S$	68.48	3.91	–	68.12	3.82	–
11o	$C_{25}H_{20}O_3S$	74.97	5.03	–	74.88	5.13	–
11p	$C_{26}H_{22}O_3S$	75.34	5.35	–	75.62	5.21	–
11q	$C_{24}H_{17}FO_2S$	74.21	4.41	–	74.23	4.70	–
11r	$C_{25}H_{19}FO_3S$	71.75	4.58	–	71.48	4.51	–
11s	$C_{26}H_{21}FO_3S$	78.09	5.24	–	77.71	4.85	–
11t	$C_{24}H_{17}NO_4S.1/2\ H_2O$	67.85	4.24	3.29	67.91	3.98	3.24
12a	$C_{24}H_{18}O_4S$	71.62	4.51	–	71.46	4.37	–
12b	$C_{25}H_{20}O_4S$	72.09	4.84	–	72.23	5.15	–
12c	$C_{26}H_{22}O_4S$	72.54	5.15	–	72.21	5.32	–
12d	$C_{25}H_{17}F_3O_4S$	63.82	3.64	–	63.55	3.82	–
12e	$C_{25}H_{20}O_5S$	69.43	4.66	–	69.05	4.36	–
12f	$C_{26}H_{22}O_5S$	69.94	4.97	–	69.73	4.61	–
12g	$C_{24}H_{17}FO_4S$	68.56	4.08	–	68.22	4.40	–
12h	$C_{25}H_{19}FO_5S$	66.66	4.25	–	66.37	4.16	–
12i	$C_{26}H_{21}FO_5S$	67.23	4.56	–	67.28	4.67	–
12j	$C_{24}H_{18}O_4S$	71.62	4.51	–	71.34	4.41	–

12k	$C_{24}H_{18}O_4S$	71.62	4.51	–	71.58	4.26	–
12l	$C_{25}H_{20}O_4S$	72.09	4.84	–	72.18	4.55	–
12m	$C_{26}H_{22}O_4S$	72.54	5.15	–	72.68	5.29	–
12n	$C_{25}H_{17}F_3O_4S.1/4H_2O$	63.16	3.68	–	63.22	3.49	–
12o	$C_{25}H_{20}O_5S$	69.43	4.66	–	69.53	4.31	–
12p	$C_{26}H_{22}O_5S$	69.94	4.97	–	69.70	4.71	–
12q	$C_{24}H_{17}FO_4S$	68.56	4.08	–	68.50	3.94	–
12r	$C_{25}H_{19}FO_5S$	66.66	4.25	–	66.28	4.51	–
12s	$C_{26}H_{21}FO_5S$	67.23	4.56	–	67.00	4.27	–
12t	$C_{25}H_{20}O_4S_2$	67.78	5.12	–	67.35	5.14	–
12u	$C_{25}H_{19}FO_4S_2$	64.36	4.10	–	64.52	4.28	–
12v	$C_{24}H_{17}NO_6S$	64.42	3.83	3.13	64.25	3.82	3.18
12w	$C_{24}H_{19}NO_4S.1/2\ H_2O$	67.53	4.69	3.28	67.28	4.48	2.98
12x	$C_{24}H_{18}O_5S$	68.88	4.34	–	68.59	4.21	–
12y	$C_{25}H_{20}O_4S_2.3/4\ H_2O$	64.92	4.65	–	64.99	4.40	–
13a	$C_{23}H_{17}NO_4S.1/4\ H_2O$	67.65	4.29	3.43	67.64	4.14	3.31
13b	$C_{24}H_{19}NO_5S$	66.50	4.42	3.23	66.17	4.28	3.14
13c	$C_{23}H_{17}NO_4S$	68.47	4.25	3.47	68.39	4.17	3.22
13d	$C_{24}H_{19}NO_5S$	66.50	4.42	3.23	66.32	4.59	3.09
13e	$C_{23}H_{17}NO_4S$	68.47	4.25	3.47	68.10	4.21	3.13
13f	$C_{24}H_{19}NO_5S$	66.50	4.42	3.23	66.43	4.25	3.34
15	$C_8H_7N_3O_2$	54.24	3.98	23.72	54.02	3.62	23.54
16	$C_{10}H_{11}N_3O_2$	58.53	5.40	20.48	58.65	5.21	20.35

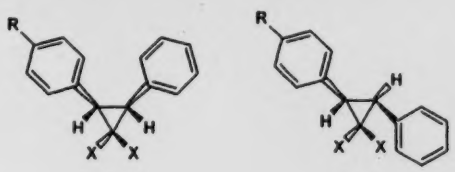
17a	$C_{24}H_{17}N_3O_3$	72.90	4.33	10.63	72.99	4.19	10.50
17b	$C_{25}H_{19}N_3O_3$	73.34	4.68	10.26	73.36	4.46	9.95
17c	$C_{24}H_{17}N_3O_2S$	70.05	4.16	10.21	69.99	3.86	10.00

6.0.0.0. GENERAL DISCUSSION AND CONCLUSIONS

Since the discovery of the inducible isoform of the enzyme cyclooxygenase-2 (COX-2), researchers across the globe have focused their attention in developing compounds that inhibit COX-2 enzyme selectively, in the hope of developing NSAIDs with a reduced side effect profile. Central ring pharmacophore templates such as diarylheterocycles have been very successful as selective COX-2 inhibitors. A common structural feature of these diarylheterocycles is the presence of 1,2-diaryl substitution on a central 4-, 5-, or 6-membered central ring system. Structure-activity relationship (SAR) studies have shown that a $-SO_2Me$, $-SO_2NH_2$ or -F substituent at the *para*-position of a phenyl ring provides optimal COX-2 selectivity, inhibitory potency and oral activity.[1] Accordingly, we have designed, synthesized and evaluated novel classes of tricyclic ring templates such as (i) 1,1-dihalo-2,3-diphenylcyclopropanes, (ii) 6-alkyl-, alkoxy- or alkylthio-3,4-diphenylpyran-2-ones, and (iii) substituted-3,4,6-triphenylpyran-2-ones. These novel ring templates, when appropriately functionalized, exhibit COX-2 selectivity and inhibitory potency along with in vivo antiinflammatory-analgesic activity.

6.1.0.0. Design, syntheses, and evaluation of novel 1,1-dihalo-2,3-diphenylcyclopropanes as potential cyclooxygenase-2 (COX-2) inhibitors with antiinflammatory-analgesic activity

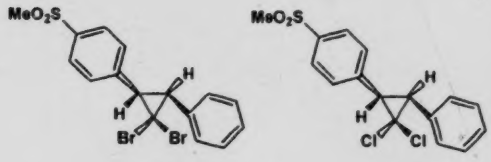
A group of (*Z*) and (*E*)-1,1-dihalo-2-(4-substituted-phenyl)-3-phenylcyclopropanes [(*Z*)-**1**, (*E*)-**2**] stereoisomers having a variety of substituents (H, Br, Cl, F, NO_2, SO_2Me) at the *para*-position of the C-2 phenyl ring in conjunction with either two chloro or bromo substituents at C-1 were synthesized and evaluated in vitro as potential selective COX-2 inhibitors, and in vivo as antiinflammatory-analgesic agents. The title (*Z*) or (*E*)-1,1-dihalocyclopropanes were prepared in good yields by the addition of dihalocarbenes ($:CX_2$), to various stilbenes. The dihalocarbenes were generated in situ either from reaction of a trihalomethane (CHX_3) in the presence of a base and a phase transfer catalyst, or from organomercurial reagents.[2-4]



R = H, Br, Cl, F, NO_2, SO_2Me

Biological evaluations for this class of compounds revealed that they exhibit good antiinflammatory-analgesic activity, but weak inhibitory activity against the cyclooxygenase isozymes. The *para*-NO_2 substituted (*E*)-isomer exhibited moderate in vitro COX-2 selectivity (COX-2 IC_{50} = 80.5 μM; COX-1 IC_{50} = 278.8 μM; Selectivity Index = 3.5). In a rat carrageenan-induced antiinflammatory assay, the potency order with respect to variation in substituents at the *para*-position of the C-2 phenyl ring for the (*Z*) series of compounds was $NO_2 > MeSO_2 \approx H \geq Cl$, and for the (*E*) series of compounds was $H \geq MeSO_2 > Cl \approx Br$. The most active

antiinflammatory agent (*E*)-1,1-dibromo-2-(4-methanesulfonylphenyl)-3-phenylcyclopropane (**3**) exhibited 45 and 37% reduction in inflammation at 3 and 5 h post drug administration, respectively relative to the reference drug celecoxib for a 50 mg/kg oral dose (80 and 58% reduction in inflammation at 3 and 5 h post drug administration for a 50 mg/ kg oral dose). In a 4% NaCl-induced writhing assay, good analgesic activity was exhibited by this group of compounds. The presence of a Cl or SO_2Me substituents at the *para*-position generally provided superior analgesic activity. The most active analgesic compound (*E*)-1,1-dichloro-2-(4-methanesulfonylphenyl)-3-phenylcyclopropane (**4**) exhibited 72 and 77% inhibition of writhing at 30 and 60 min post drug administration (50 mg/kg ip route).



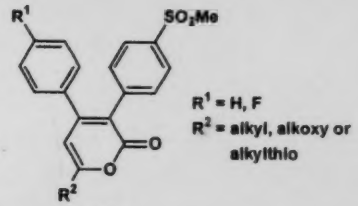
(*E*)-3 (*E*)-4

Molecular modeling studies for some of the compounds in this series showed that (*E*)-1,1-dihalo-2-(4-substituted-phenyl)-3-phenylcyclopropanes (**2**) generally bind more favourably within the COX-2 active site compared to the (*Z*)-isomers. This explains the inactive nature of (Z)-isomers as COX inhibitors.[5]

In conclusion, the results of this investigation shows that (i) a 3-membered cyclopropane ring can serve as a potential ring template in the design of tricyclic COX-2 inhibitors that exhibit in vivo antiinflammatory-analgesic activity and that (ii) the COX-2 binding interaction is governed by steric and electronic properties at the *para*-position on the C-2 phenyl ring, (iii) molecular modeling studies for this class of compounds reveal that favorable binding interaction within the COX-2 active site may be obtained by placing appropriate dihalo substituents at the C-1 position of the cyclopropane ring.

6.2.0.0. Design, synthesis and biological evaluation of 6-alkyl-, alkoxy- or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones: A novel class of diarylheterocyclic selective cyclooxygenase-2 inhibitors

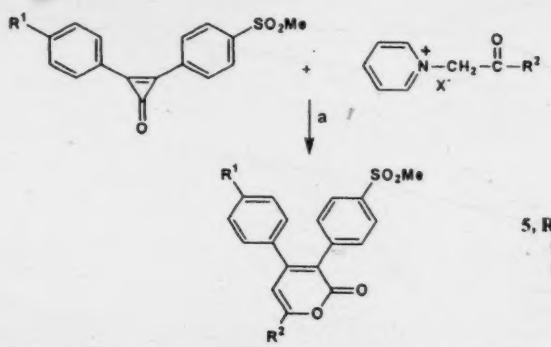
A group of 3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones (**5**) possessing a central six-membered lactone (pyran-2-one) ring was designed as a novel COX-2 selective central ring template. The substituent at the C-6 position of the central lactone was varied (alkyl, alkoxy and alkylthio) to determine their effect on in vitro COX-2 selectivity, inhibitory potency and in vivo antiinflammatory-analgesic activity.



(5)

The 3,4-diarylpyran-2-ones (**5**) were prepared in low to moderate yields (8–44%) by condensation of a 2-(4-methanesulfonylphenyl)-3-phenylcycloprop-2-en-1-one with a pyridinium salt in the presence of the base triethylamine (Scheme 6.1). The

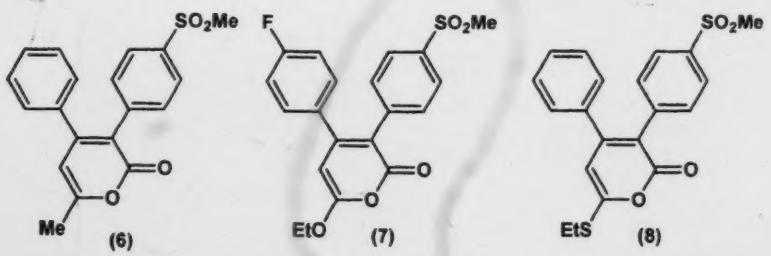
initial yilde compound that is formed in the reaction undergoes a spontaneous ring expansion reaction to produce the title compounds having a central six-membered lactone ring (pyran-2-one).[6,7] No other regioisomer was observed using the reaction conditions employed. ^{1}H NMR nuclear Overhauser enhancement (NOE) studies showed NOE interactions between H-5 and the 6-alkyl (R^2), alkoxy (OR^2), or alkylthio (SR^2) moiety, and between H-5 and the C-4 *o*-phenyl hydrogens. This established the regiochemistry of the C-4 and C-5 phenyl rings. The starting material, 2-(4-methanesulfonylphenyl)-3-phenylcycloprop-2-en-1-one, was prepared using a one-pot reaction according to our previously reported procedure.[8]



5, R^1 = H, F; R^2 = Me, Et, *i*-Pr, OMe, OEt, O-*i*-Pr, O-*t*-Bu, O-*i*-Bu, O-CH_2-C_6H_4-4-Cl, SMe, SEt, S-*n*-Pr, S-*i*-Pr

Scheme 6.1: Reagents and conditions: (a) benzene, Et_3N, 25 °C, 16–18 h.

In vitro COX enzyme inhibition studies for the C-6 alkyl (R^2 = Me, Et, *i*-Pr) subgroup of compounds (**5**) showed weak to good inhibitory activity, with 6-methyl-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (**6**) showing the best combination of COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.68 μM ; COX-1 IC_{50} = 614.8 μM; SI = 904). For this subgroup of compounds, a *p*-fluoro substituent on the C-4 phenyl (R^1 = F), generally decreased COX-2 inhibitory potency and selectivity. For the C-6 alkoxy (R^2 = OMe, OEt, OPr-*i*, OBu-*t*, OBu-*i*, OCH_2-C_6H_4-4-Cl) subgroup, the COX-2 selectivity and potency may be dependent on the steric effect as a reduced COX-2 inhibitory potency and selectivity was observed with the introduction of a C-6 4-chlorobenzyloxy substituent (R^2 = OCH_2-C_6H_4-4-Cl).



The most active compound in this sub group was (**7**, COX-2 IC_{50} = 0.10 μM; COX-1 IC_{50} = 288 μM; SI = 2880), which possessed a C-6 ethoxy (R^2 = OEt) and a *p*-fluoro substituent on the C-4 phenyl ring (R^1 = F). Among the C-6 alkylthio (MeS, EtS, *n*-PrS, *i*-PrS) subgroup, the COX-2 inhibitory potency was of the order EtS >> MeS > inactive *n*-PrS and *i*-PrS. The C-6 thioethyl compound (**8**) was a highly potent and selective COX-2 inhibitor (COX-2 IC_{50} = 0.003 μM; COX-1 IC_{50} = 382.6 μM; SI > 120,000) that was a much more potent and selective inhibitor than the reference drugs (celecoxib COX-2 IC_{50} = 0.05 μM; SI = 401; rofecoxib COX-2 IC_{50} = 0.43 μM; SI > 1162).[9]

A molecular modeling (docking) study was carried out to investigate the binding interactions of (**7**) within the COX-2 active site.[10] These docking studies showed that the COX-2 *p*-SO_2Me pharmacophore was oriented favourably within the COX-2 active site where it interacted with amino acids lining the COX-2 secondary pocket (His^{90}, Gln^{192}, Arg^{513}, Phe^{518}, Val^{523} and Leu^{352}). The *p*-SO_2Me was inserted deep inside the COX-2 secondary pocket with the *O*-atoms of SO_2Me forming a hydrogen bonding interaction with Phe^{518}. The central pyran-2-one ring was oriented towards the mouth of the channel (Tyr^{355} and Arg^{120}). The C-4 *p*-fluorophenyl ring was oriented towards a hydrophobic pocket made up of Trp^{387}, Tyr^{385} and Tyr^{348}). Interestingly, the C-6 ethoxy substituent was oriented towards a hydrophobic region comprised of Val^{349}, Ile^{345}, Ser^{530}, Leu^{531} and Met^{535}. Therefore, for this class of compounds, the orientation of the *p*-SO_2Me pharmacophore was dependent on the substituent electronic and steric effects at the C-6 position of the central pyran-2-one. A similar docking study of (**7**) within the COX-1 active site showed that the presence of a bulkier Ile (Val in COX-2) at position 523 prevents the access of COX-2 SO_2Me pharmacophore to a side pocket. This explains the potent COX-2 (IC_{50} = 0.10 μM) and weak COX-1 (IC_{50} = 288 μM) inhibitory activity of (**7**). When a docking experiment was carried out for the most active compound (**8**, C-6 EtS), the binding interactions were similar to those observed for the C-6 ethoxy analog (**7**) within the COX-2 active site. The *p*-SO_2Me COX-2 pharmacophore was inserted deep inside within the COX-2 secondary pocket. The C=*O* of the central pyran-2-one forms a hydrogen bonding with Tyr^{355} at the mouth of the COX-2 channel. The C-6 SEt was oriented towards a hydrophobic pocket where the sulfur atom undergoes a weak hydrogen bonding interaction with the O*H* of Ser^{530}. Recent studies have shown the importance of Ser^{530} in the COX-2 inhibitory potency of diarylheterocyclic COX-2 inhibitors.[11] These docking experiments further confirm the role of C-6 substituents that induce the proper orientation of the *p*-SO_2Me pharmacophore within the COX-2 active site.

In vivo antiinflammatory-analgesic screening for this pyran-2-one class of compounds showed that they exhibit a wide range of activities. Compound (**7**) exhibited good antiinflammatory activity at different time intervals (32 and 67% reduction in inflammation at 3 and 5 h post drug administration) for a 1 mg/kg oral dose, which parallels its potent in vitro COX-2 inhibitory activity (COX-2 IC_{50} = 0.10 μM; SI = 2880). In contrast, the most

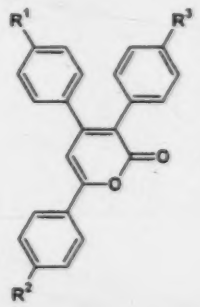
potent and selective COX-2 inhibitor (**8**, COX-2 IC_{50} = 0.003 μM; SI > 120,000) was a weak antiinflammatory agent (9 and 19.5% reduction in inflammation at 3 and 5 h post drug administration) for a 50 mg/kg oral dose. In a 4% NaCl-induced writhing assay, (**7**) exhibited good analgesic activity (42 and 75% inhibition in writhing at 30 and 60 min. respectively, post drug administration) for a 1 mg/kg intraperitoneal dose. The C-6 SEt compound (**8**) was the most active analgesic agent (exhibited 81 and 77% inhibition of writhing at 30 and 60 min. respectively, post drug administration) for a 50 mg/kg intraperitoneal dose.

In conclusion, qualitative structure-activity data acquired for this class of 6-alkyl-, alkoxy-, or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones have shown that (i) a six-membered lactone (pyran-2-one) ring serves as a suitable central ring template to design selective COX-2 inhibitors, (ii) the combined electronic and steric properties at the C-6 position of the central lactone ring modulates COX-2 inhibitory potency and selectivity by orienting the C-3 *p*-SO_2Me phenyl substituent to the vicinity of the secondary pocket within the COX-2 binding site, and (iii) for this class of compounds, the in vivo antiinflammatory activity was improved by the presence of a *p*-fluoro substituent on the C-4 phenyl ring.

6.3.0.0. Design, synthesis and structure-activity relationship (SAR) studies of 3,4,6-triphenylpyran-2-ones as selective cyclooxygenase-2 inhibitors

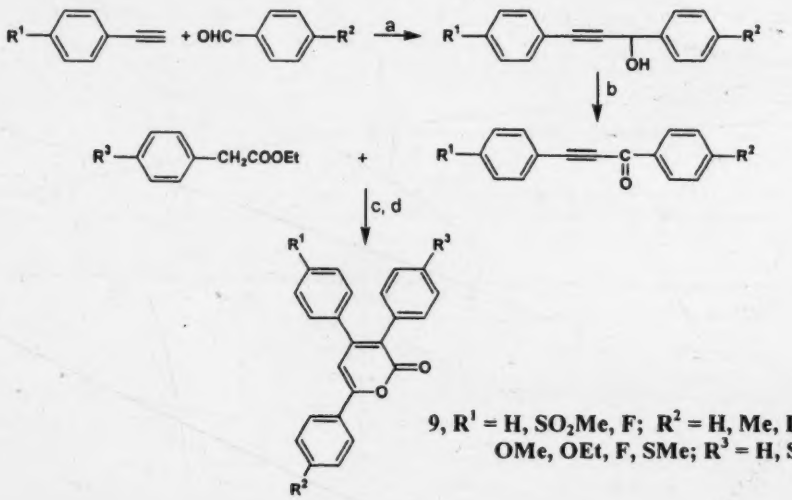
As an extension of our previous work[10] we designed and synthesized a new group of regioisomeric 3,4,6-triphenylpyran-2-ones (**9**) that possessed a *p*-SO_2Me pharmacophore at either the C-3 or C-4 phenyl ring of the central pyran-2-one, in conjunction with a C-6 *p*-substituted phenyl ring. For this class of compounds, SAR data were acquired in vitro (IC_{50} values), to assess the effect of regioisomeric placement of the *p*-SO_2Me pharmacophore (at either C-3 or C-4 phenyl) and the role of *para*-substituted phenyl ring at the C-6 position of the central pyran-2-one, on COX-2 inhibitory potency and selectivity. In vivo antiinflammatory-analgesic activity was assessed using carrageenan-induced rat paw edema and 4% NaCl-induced abdominal assay animal models.

Synthesis of the title compounds was achieved by a series of reactions starting from the condensation of appropriately substituted phenylacetylenes with various *p*-substituted benzaldehydes in the presence of *n*-BuLi to afford respective 1,3-diarylprop-2-yn-1-ols,[12] which were subsequently oxidized to the respective 1,3-diarylprop-2-yn-1-ones. The final cyclization was achieved by reaction of the respective 1,3-diarylprop-2-yn-1-one with the *para*-substituted-phenylacetic acid ester in the presence of a base (NaH or potassium-*t*-butoxide).



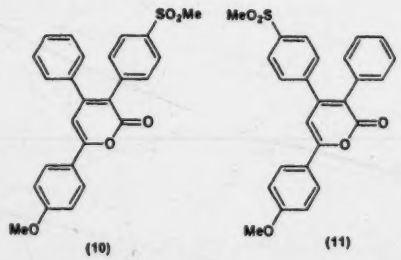
9, R^1 = H, SO_2Me, F; R^2 = H, Me, Et, CF_3, OMe, OEt, F, SMe; R^3 = H, SO_2Me, F, N_3

Oxone® oxidation afforded the respective title compounds (Scheme 6.2).



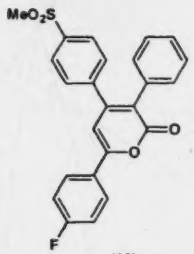
Scheme 6.2: Reagents and conditions: (a) THF, -78 °C, *n*-BuLi, and then at -78 °C to 25 °C over night; (b) acetone, MnO_2, 25 °C, 4–5 h; (c) DMSO, NaH, 25 °C, 1 h; (d) 1,4-dioxane, aqueous Oxone®, 25 °C, 4–5 h.

The in vitro ability of this class of compounds to inhibit the enzymes COX-1 and COX-2 (IC_{50} values) was evaluated, and the most potent and COX-2 selective compounds were evaluated in vivo to determine their antiinflammatory-analgesic activities.[10,13,14] For the C-3 *p*-SO_2Me-phenyl regioisomers, varying the substituents at the *para*-position of the C-6 phenyl ring had a dramatic effect on COX-2 inhibitory potency and selectivity, with compound (**10**) possessing a C-6 *para*-methoxyphenyl substituent exhibiting excellent COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.02 μM; COX-1 IC_{50} > 100 μM; SI > 5000). Compound (**10**) was 3.5- and 25-fold more potent than celecoxib (COX-2 IC_{50} = 0.07 μM; SI = 474) and rofecoxib (COX-2 IC_{50} = 0.50 μM; SI > 200), respectively. Introduction of a fluorine substituent at the *para*-position on the C-4 phenyl ring generally decreased COX-2 inhibitory potency and selectivity. For this group of C-3 *p*-SO_2Me-phenylpyran-2-ones, the relative COX-2 inhibitory potency order was OMe > OEt > SMe > NH_2 > F > NO_2 > inactive H, Me, Et, CF_3 and OH.



The C-4 *para*-methanesulfonylphenyl regioisomer (**11**), which possesses a C-6 *para*-methoxyphenyl substituent, exhibited good COX-2 inhibitory potency (COX-2

IC_{50} = 0.45 μM; SI = 70), but it was less potent and selective than the corresponding C-3 *para*-methanesulfonylphenyl regioisomer (**10**). Replacement of the C-6 *p*-methoxyphenyl substituent with a *p*-fluorophenyl substituent gave a highly potent COX-2 inhibitor (**12**, COX-2 IC_{50} = 0.07; SI = 157). Among the C-3 or C-4 pyridyl substituted pyran-2-ones, 3-(pyridin-4-yl)-4-(4-methanesulfonyl phenyl)-6-(4-methoxyphenyl)pyran-2-one (**13**) exhibited the best combination of COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.32 μM; SI > 312.0).



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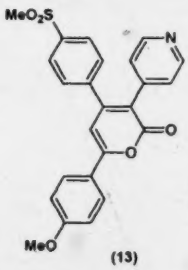
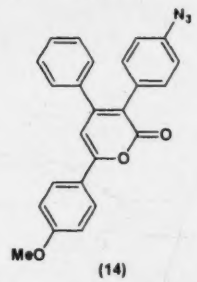
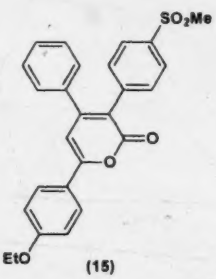


(13)

Recently we exploited, for the first time, the amino acid Arg^{513} to design selective COX-2 inhibitors having a dipolar azide (N_3) pharmacophore that can undergo an electrostatic (ion-ion) interaction with Arg^{513} in the COX-2 2°-pocket.[15] Accordingly, we replaced the SO_2Me pharmacophore in the most potent and selective COX-2 inhibitors identified from the 3,4,6-triphenylpyran-2-ones investigated in this study with a dipolar azido bioisostere (**14**). In this regard, these compounds retained their COX-2 selectivity, but were less potent than their corresponding C-3 *p*-SO_2Me-phenyl analogs, with (**14**) exhibiting good COX-2 inhibitory potency and selectivity (COX-2 IC_{50} = 0.5 μM; COX-1 IC_{50} > 100 μM; SI > 200) among this subgroup of compounds.



(14)



(15)

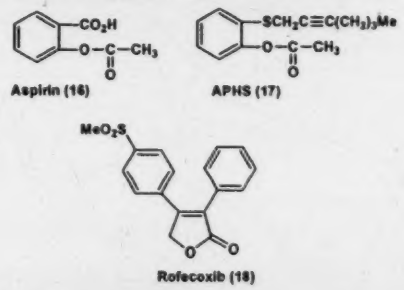
The binding mode of compounds **10**, **11** and **14** within the COX-2 active site was investigated by a series of molecular modeling (docking) studies. As expected, in case of the pyran-2-one regioisomers **10** and **11**, the *p*-SO_2Me pharmacophore was interacting with the amino acid residues lining the COX-2 secondary pocket. However, due to the regiochemistry, the C-6 *para*-substituted-phenyl ring and the C=O of the central pyran-2-one ring were oriented in different regions within the COX-2 active site. In the case of **10**, the C-6 *p*-methoxyphenyl ring was oriented towards a hydrophobic pocket close to the mouth of the COX-2 active site, and was within a van der Waal's contact range (distance ≈ 5 Å) of Ala^{527}, Ser^{530}, Leu^{531}, Met^{535}, Ile^{345}, Val^{349}, and Leu^{359}. In addition, the central 6-membered lactone (pyran-2-one) ring was located near the mouth of the COX-2 binding site such that, the central C=*O* of the lactone ring undergoes a weak hydrogen bonding interaction with the O*H* of Ser^{353} (distance = 2.91 Å). Therefore, the OMe substituent may be responsible for proper orientation of the C-3 *p*-SO_2Me-phenyl ring within the COX-2 secondary pocket (Gln^{192}, Arg^{513} and Phe^{518}). In contrast, the C-6 *p*-MeO-phenyl moiety of **11** was located near the mouth of the COX-2

binding site, and was surrounded by amino acid residues Met^{113}, Val^{116}, Arg^{120}, Val^{349}, Tyr^{355}, Phe^{357} and Leu^{359}, and the OMe substituent was much further removed from Met^{535} compared to the C-3 *p*-$MeSO_2$-phenyl regioisomer **10**. In addition, a crucial hydrogen bonding interaction between the backbone of Phe^{518} and one of the *O*-atoms of *p*-SO_2Me pharmacophore, seen within the COX-2 secondary pocket for **10,** was not observed with **11**. This may explain, the higher COX-2 inhibitory potency exhibited by **10** (COX-2 IC_{50} = 0.02 μM; SI > 5000) as compared to **11** (COX-2 IC_{50} = 0.45 μM; SI = 70). A docking experiment of the pyran-2-one **14**, within the COX-2 active site, in which the SO_2Me pharmacophore was replaced by a linear azido (N_3) bioisostere, was similar to that of the respective C-3 *para*-$MeSO_2$-phenyl analog **10**. As per our hypothesis, the dipolar azido group was interacting with secondary pocket amino acids (Phe^{518}, Arg^{513} and His^{90}) and undergoes a favourable interaction with NH_2 (guanidino group) of Arg^{513} (ion-ion interaction). These observations show that the dipolar azido pharmacophore serves as a suitable bioisostere that undergoes electrostatic interaction with Arg^{513} within the COX-2 secondary pocket.

In the in vivo carrageenan-rat paw edema assay, the most active oral antiinflammatory compound was **15** (COX-2 IC_{50} = 0.05 μM; S.I. > 2000) which, exhibited an ID_{50} of 5.6 mg/kg and was more potent than the reference drug celecoxib (ID_{50} = 10.8 mg/kg). In a rat model 4% NaCl-induced abdominal constriction assay, the 6-(4-ethoxyphenyl)-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-one (**15**) exhibited good analgesic activity where a 5 mg/kg po dose reduced writhing by 58 and 75% at 30 and 60 min post drug administration.

In conclusion, the SAR data acquired for this class of 3,4,6-triphenylpyran-2-ones have shown that (i) compounds exhibiting high COX-2 inhibitory potency and selectivity can be designed by appropriate placement of a *p*-SO_2Me pharmacophore on either a C-3 or C-4 phenyl ring, of the central the pyran-2-one ring, (ii) the C-3 *p*-$MeSO_2$-phenyl regioisomer having an appropriately substituted C-6 phenyl ring, exhibits better binding affinity than the corresponding C-4 *p*-$MeSO_2$-phenyl regioisomer, (iii) COX-2 inhibitory potency and selectivity are sensitive to substituent electronic properties at the *para*-position of the C-6 phenyl ring where the C-6 *p*-MeO-phenyl (**10**) exhibits the best combination of potency and selectivity, and (iv) the linear azido (N_3) substituent is a suitable bioisostere to replace a traditional SO_2Me COX-2 pharmacophore.[16]

6.4.0.0. Molecular modeling studies of isomeric acetoxy analogs of rofecoxib

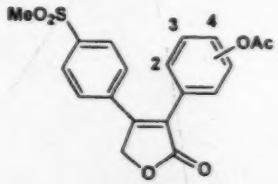


Aspirin (**16**) is a unique non-selective COX inhibitor due to its ability to acetylate the serine hydroxyl group in the COX binding site of COX-1 and COX-2. In this regard, aspirin is a 10- to 100-fold more potent inhibitor of COX-1

relative to COX-2.[17] Some of aspirin's beneficial therapeutic effects can be attributed to acetylation of COX-2, while its antithrombotic and ulcerogenic effects are due to acetylation of COX-1. These observations were elegantly exploited in the design of the aspirin analog *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS, **17**) that selectively acetylated, and irreversibly inactivated, COX-2.[18] Rofecoxib (**18**) is a highly potent and selective COX-2 inhibitor widely used clinically. As part of our ongoing program, we designed and synthesized a group of isomers possessing a 2-, 3- or 4-acetoxy moiety on the C-3 phenyl substituent of rofecoxib (**19a–c**), that exhibited highly potent and selective, COX-2 inhibitory activity (Table 6.1).[19]

site, with the SO_2Me pharmacophore interacting with the secondary pocket amino acid residues (Phe^{518}, Arg^{513}, Gln^{192} and His^{90}) and the central lactone ring oriented towards the mouth of the COX-2 active site (Tyr^{355} and Arg^{120}). The C-3 phenyl ring was oriented towards a hydrophobic pocket made up of Trp^{387}, Tyr^{385} and Tyr^{348}. As per our hypothesis, the acetoxy substituent of all three isomers **19a–c** was oriented in the vicinity of Ser^{530}. However, the orientation of the C-3 *p*-acetoxy substituent was different for all three isomers with respect to the distance between the carbon atom of the *C*=O (of the acetoxy substituent) and the *O*H of Ser^{530}. The rofecoxib analog **19c**, which had a acetoxy substituent at the *para*-position of the C-3 phenyl ring, was

Table 6.1: In vitro inhibition of COX-1 and COX-2 by 3-(2-, 3- and 4-acetoxyphenyl) analogs of rofecoxib (**19a–c**)



19a (2-OAc)
19b (3-OAc)
19c (4-OAc)

Compd	COX-1 inhibition IC_{50}, μM	COX-1 inhibition IC_{50}, μM	COX-2 Selectivity Index
19a	> 100	0.0035	> 28,000
19b	> 100	0.0017	> 59,000
19c	> 100	0.0013	> 79,000

The potential of **19a–c** to acetylate the COX-2 isozyme was studied by a series of docking experiments. These isomeric rofecoxib analogs bind in a similar way within the COX-2 active site, with the SO_2Me pharmacophore interacting very favourably within the COX-2 active site, with the *C*=O (of the *p*-acetoxy substituent) almost 5.76 Å away from *O*H of Ser^{530} (Figure 6.1). It was interesting to note that the *O*-atom of

the C=*O* (*p*-OAc) forms a hydrogen bond with the O*H* of Tyr^{385} (distance = 1.84 Å), which could potentially activate the *C*-atom of the acetoxy moiety with respect to nucleophilic attack by the *O*H of Ser^{530} that may lead to a covalent acetylation of the COX-2 isozyme. This observation is consistent with the critical role of Tyr^{385} in the acetylation of Ser^{530} by aspirin in the COX binding site.[20] These observations, explain the potent and selective COX-2 inhibition (COX-2

6.5.0.0. Molecular modeling studies of celecoxib analogs

Development of the non-ulcerogenic selective COX-2 inhibitor celecoxib (**20**), provided a significant advancement in the treatment of RA and OA. The SO_2NH_2 pharmacophore present in celecoxib is believed to induce COX-2 selectivity by insertion into the secondary pocket of the COX-2 binding site that is absent in COX-1. It has been reported

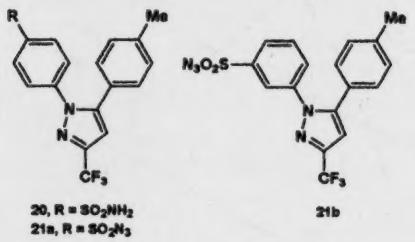
Figure 6.1: Docking of the rofecoxib analog (**19c**, ball and stick) in the active site of murine COX-2.

IC_{50} = 0.0013 μM; SI > 79,000) exhibited by **19c**.

In conclusion, results of this investigation show that (i) incorporation of a 2-, 3-, or 4-OAc substituent on the C-3 phenyl ring of rofecoxib provides highly potent, and selective, COX-2 inhibitors, (ii) molecular modeling studies help in predicting the acetylating potential of the isomeric rofecoxib analogs, and (iii) the acetoxy compounds could serve as useful probes to study the function and catalytic activity of the COX-2 isozyme.

that replacement of His^{513} in COX-1 by Arg^{513} in COX-2 plays a key role in the hydrogen-bond network of the COX-2 binding site. Access of ligands to the secondary-pocket of COX-2 is controlled by histidine (His^{90}), glutamine (Gln^{192}) and tyrosine (Tyr^{355})[21], and interaction of Arg^{513} with the bound drug is a requirement for time-dependent inhibition of COX-2.[22] Recently we exploited, for the first time, the amino acid Arg^{513} to design selective COX-2 inhibitors having a dipolar azide (N_3) pharmacophore that can undergo an electrostatic (ion-ion) interaction with Arg^{513} in the COX-2 secondary pocket.[15] Accordingly, we designed and

synthesized novel celecoxib analogs (**21a** and **21b**) in which the *p*-SO_2NH_2 pharmacophore was replaced by a novel dipolar sulfonylazide (SO_2N_3) pharmacophore that has the potential to undergo dual *H*-bonding (sulfonyl oxygens) and electrostatic (ion-ion) interactions (azido) with amino acid residues lining the secondary-pocket of the COX-2 binding site.



A molecular modeling study of the celecoxib analog 4-[5-(4-methylphenyl)-3-trifluoromethyl-1*H*-pyrazol-1-yl]benzenesulfonylazide (**21a**) docked in the active site of the murine COX-2 isozyme showed **21a** binds in the center of the enzyme active site, with the phenylsulfonylazide moiety oriented towards the mouth of the COX-2 channel such that the SO_2N_3 group is in close proximity with Arg^{120} and Tyr^{355} (Figure 6.2). The linear N_3 group is involved in extensive electrostatic interaction with the side chains of Arg^{120} and Tyr^{355}. Llorens and coworkers have shown the importance of the perturbation of the hydrogen bonding network involving Arg^{120}, Glu^{524}, Tyr^{355} and His/Arg^{513} at the mouth of the channel by different ligands and their effect on COX inhibition.[21] The SO_2N_3 terminal *N*-atom

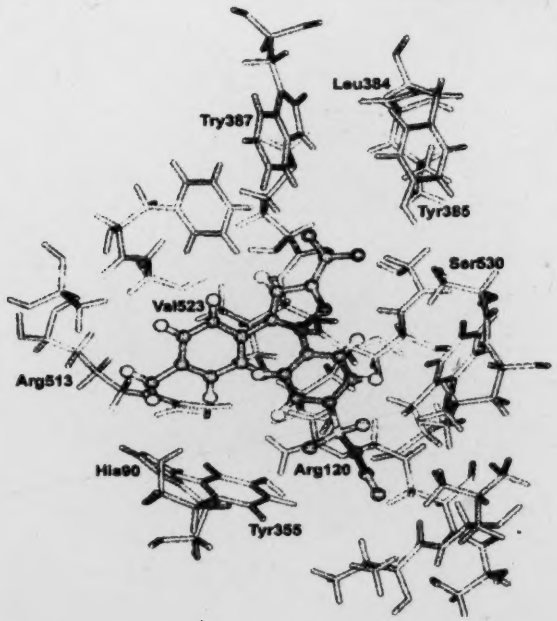


Figure 6.2: Docking of the celecoxib analog (**21a**, ball and stick) in the active site of murine COX-2.

is located about 4.93 Å from the O*H* of Tyr^{355}, a SO_2 *O*-atom of the SO_2N_3 substituent is about 4.30 Å away from the O*H* of Tyr^{355}, and the C-5 phenyl ring is oriented toward Val^{523}. The C-3 CF_3 substituent is oriented towards a hydrophobic pocket comprised of Leu^{384}, Tyr^{385} and Trp^{387}, and it is positioned about 3.32 Å from the O*H* of Tyr^{385} and about 4.92 Å from the O*H* of Ser^{530}. The N^2-atom of the central pyrazole ring is about 5.88 Å away from the O*H* of Ser^{530}. Recent studies have shown the importance of ionic interactions involving the CO_2H group of NSAIDs with Arg^{120} and its critical role in COX-1 inhibition.[23,24] The in vitro COX inhibition data showing that **21a** is a selective COX-1 inhibitor (COX-1 IC_{50} = 3.3 μM; COX-2 IC_{50} > 100 μM) are consistent with this docking experiment which indicates that the dipolar N_3 moiety of the SO_2N_3 substituent is interacting with Arg^{120} at the mouth of the primary binding site, like the CO_2H group of NSAIDs, rather than insertion into the secondary-pocket of the COX-2 isozyme near Val^{523} that is required for selective COX-2 inhibition.

In contrast, 3-[5-(4-methylphenyl)-3-trifluoromethylpyrazol-1-yl) benzenesulfonylazide (**21b**) which has a *meta*-SO_2N_3 substituent at the N^1-phenyl ring, binds in the center of the active site with the phenylsulfonyl azide moiety oriented towards the secondary-pocket region of the COX-2 binding site (Figure 6.3). The terminal *N*-atom of the *m*-SO_2N_3 is inserted about 4.25 Å deep inside the entrance to the secondary-pocket of COX-2 (Val^{523}) and about 5.20 Å removed (within electrostatic ion-ion interaction distance) from the NH_2 of Arg^{513}. A SO_2 oxygen atom of the SO_2N_3

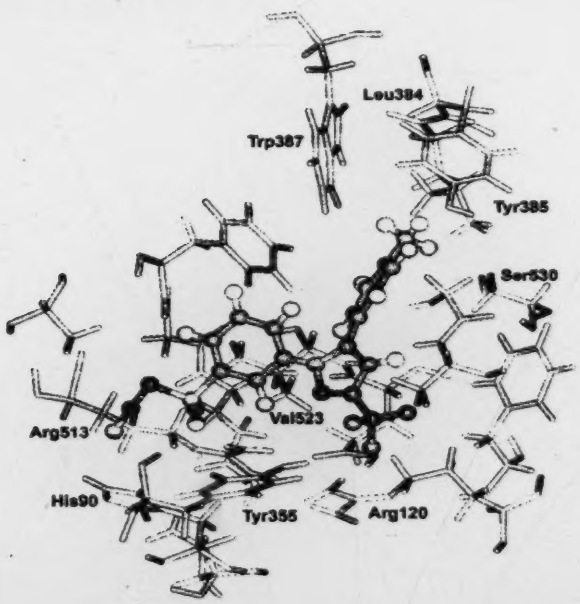


Figure 6.3 Docking of the celecoxib analog (**21b**, ball and stick) in the active site of murine COX-2.

group is about 2.74 Å away (within *H*-bonding distance) from the NH_2 of Arg^{513}. The C-3 CF_3 substituent is positioned about 5.63 Å from the NH_2 of Arg^{120} and the N^2-nitrogen atom of the central pyrazole ring is located about 3.11 Å from the O*H* of Tyr^{355}. The center of the C-5 phenyl ring is about 5.87 Å from the *O*H of Ser^{530} with the Me group at the *para*-position orienting in a hydrophobic region surrounded by Leu^{384}, Tyr^{385} and Trp^{387} binding affinity. Accordingly, **21b** was a selective COX-2 inhibitor (COX-2 IC_{50} = 5.16 μM; COX-1 IC_{50} > 100 μM) with a COX-2 selectivity index > 19.

Results of this molecular modeling (docking) studies showed that (i) the sulfonylazido (SO_2N_3) group serves as a suitable COX-2 pharmacophore in the design of selective COX-2 inhibitors, and (ii) when suitably placed, the SO_2N_3 group interacts within the secondary-pocket of the COX-2 isozyme where it can undergo both *H*-bonding via one of its SO_2 oxygen-atoms, and an electrostatic ion-ion interaction via its terminal azide nitrogen atom, with the guanidino NH_2 group of Arg^{513}.[25]

6.6.0.0 Conclusions

A diverse variety of tricyclic COX-2 inhibitors belonging to the diarylcycloalkyl (1,1-dihalo-2,3-diphenyl cyclopropanes) and diarylheterocyclic [6-alkyl-, alkoxy- or alkylthio-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones, substituted-3,4,6-triphenylpyran-2-ones, 3,4-diarylfuranones and 1,5-diarylpyrazoles) classes can be designed successfully using rational drug design concepts in conjunction with molecular modeling (docking) studies, chemical synthesis, and acquisition of the in vitro and in vivo structure-activity relationship (SAR) data.

6.7.0.0 REFERENCES

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